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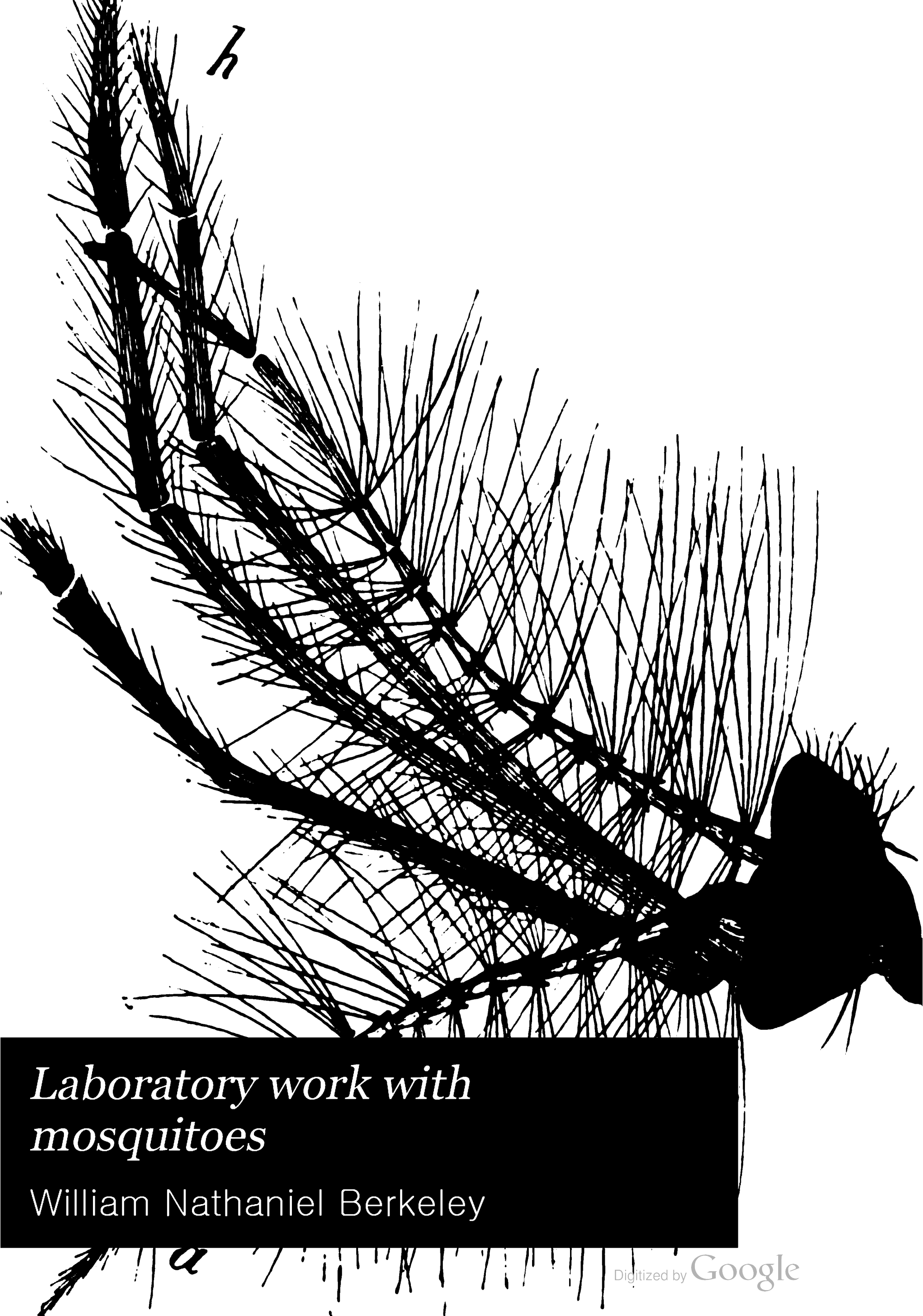

Laboratory work with mosquitoes

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LABORATORY WORK WITH MOSQUITOES

BY

WILLIAM N. BERKELEY, A.B., M.D.,
NEW YORK.

WITH 65 ILLUSTRATIONS, LARGELY ORIGINAL.

NEW YORK.
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PREFACE.

Besides personal acknowledgments in the text, my sincere thanks are due to Miss Marion Satterlee and Dr. Louise Cordes for the devotion and care they have given the illustrations, in the making of which no pains have been spared to reproduce faithfully the objects drawn.

The laboratory facilities of the Pediatrics Laboratory and of the Presbyterian Hospital, and the clinical material of the Third Division of Bellevue Hospital have been placed at my disposal ever since I began, some years ago, the studies this book is based on. Without the help thus generously extended I should have been able to accomplish nothing.

Messrs. William Wood and Company, of New York, have courteously loaned the plates of several drawings first appearing in an article of mine in the *Medical Record* for December 23, 1899. These illustrations are individually acknowledged as they occur.

Of the chapter on *Stegomyia* Theobald both the text and illustrations have been furnished by Dr. Aristides Agramonte, U.S. Army Medical Service, Havana, Cuba. As the first authoritative and complete account of *Stegomyia fasciata* so far published I believe this chapter to be of peculiar value.

The key to the *Culicidæ* is virtually a reprint of Mr. D. W. Coquillett's contribution to Dr. L. O. Howard's recent work, *Mosquitoes*. It is reproduced here with the consent of the gentlemen named, to whom I must record, both for this and many other courtesies, my deep obligations. This book is meant in a measure to be a laboratory supplement to Dr. Howard's more popular and extensive volume.

My own purpose has been to give only the information needed for successful experiment in the laboratory upon the *Culicidæ* as the hosts or possible hosts of parasites having an additional life-cycle in man or other vertebrates. I may scarcely hope that amid such a multitude of difficult and minute details mistakes of greater or less gravity have not crept in. For such, as many as there be, I can only beg the reader's indulgence.

If excuse for this diffident effort be required, I cannot offer it in better words than those of Dr. Patrick Manson, to whose genius and patience humanity owes perhaps as large a debt of gratitude as to that of any physician in the last hundred years:—

"Full knowledge of all that concerns the etiology of malaria will only be attained when we have full knowledge of the various species of mosquito capable of subserving the germ, of certain vertebrates which may be capable of taking the place of man in the malarial cycle, of their geographical distribution, of their habits, and of their enemies. As yet this knowledge is but beginning. . . . Whether certain species of Culex may not be efficient hosts for the Plasmodium, as they certainly are for Proteosoma and Filaria nocturna, can as yet be neither affirmed nor denied. Studies in this field are being actively carried on, so that in a very few years important additions to our knowledge of great practical value may be confidently expected."

W. N. B.

121 East Twenty-sixth Street.
January, 1902.

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LABORATORY WORK WITH MOSQUITOES

CHAPTER I.

DEVELOPMENT AND MODE OF LIFE OF THE CULICIDÆ.

CULEX.

The developmental cycle of the numerous species in this genus is fairly typified by **Culex pungens** Wied., and may be briefly described as follows:

The **eggs** are laid upon water. They are minute ovoid bodies, 0.6 to 0.8 mm. long, larger at one end, black in color after exposure to air and usually grouped by lateral adhesion into small masses that float on the surface. The axis of the eggs is vertical and the larger end points down. Several hundred eggs are said to be commonly laid in one mass.

The **larva** (the common "wriggler") emerges in from 16 hours (1)* to 3 days after oviposition,—according to temperature. It escapes from the larger (lower) end of the egg, and begins at once an active life in pursuit of food. The figure (Fig. 1) will supply all needful description. It should be specially noted that the breathing tube or siphon (projecting upward from the penultimate abdominal ring) is thick and long, and that the head of the larva hangs well below the surface of the water, the long axis of the body being more nearly vertical than horizontal. Habitually, therefore,

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* Numerals in parenthesis refer to the List of Authorities in the back of the book.

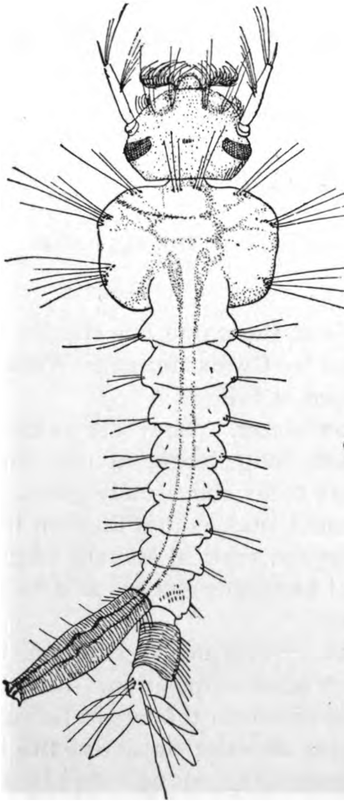

Fig. 1.—Full-grown larva of *Culex pungens*; enlarged. (From Howard).

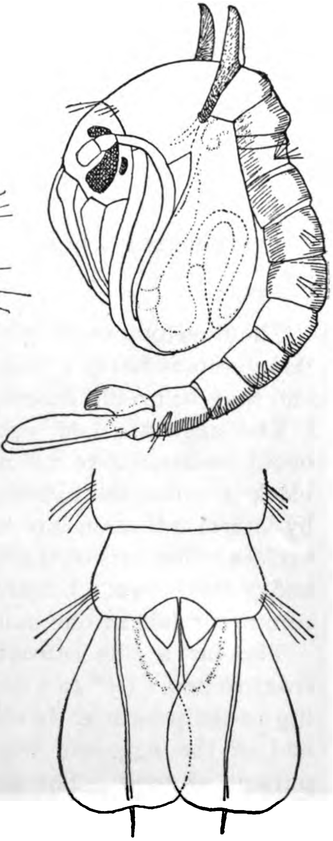

Fig. 2.—Pupa of *Culex pungens*; enlarged. Anal segment below still more enlarged. Breathing siphons project upward from back part of thorax. (From Howard).

the insects feeds *below* the surface. The head may occasionally be raised to the surface film to catch a morsel of food or bite off a particle which obstructs the air-tube, but if so, the body is bent on itself, never lying flat on the surface. If disturbed the larva wriggles to the bottom of the pool or vessel and hides, but returns usually within a minute or two to the surface for air. During development the larva moults several times. The minimum period of larval life under favoring conditions is about 7 days.

The **pupa** (Fig. 2) moves actively but does not eat. When frightened it descends to the bottom by awkward and violent movements of the tail, which is tipped with two broad flaps. In very shallow water or wet mud it survives for some time with one siphon in the air and the other in the mud. Laid on a dry surface it jumps violently, its movements suggesting those of a minute frog. It develops rapidly, and in 36 to 48 hours in warm weather the pupa-case is split above, and the **imago** or adult insect appears. The differences between the larva and pupa of Culex and Anopheles will be noted later (p. 8). The males usually hatch first, and are ordinarily more numerous than the females.

An adult female Culex (*C. pungens* Wied.) is sketched in Fig. 3. The generic marks as a whole are readily recognized with the naked eye. The *palpi* in the *female* are much *shorter*, in the *male* somewhat *longer*, than the proboscis (Figs. 4, 5). In the male the palpi sometimes curl up at their tips. The *posture* of the live insect on a vertical wall is also characteristic, the axis of the body inclining so that the tip of the tail is a little closer to the wall than the head and thorax are, while the last pair of legs is extended backward above the body. This is notably different from the posture of Anopheles. The illustrations on pp. 15-17 explain this perfectly.

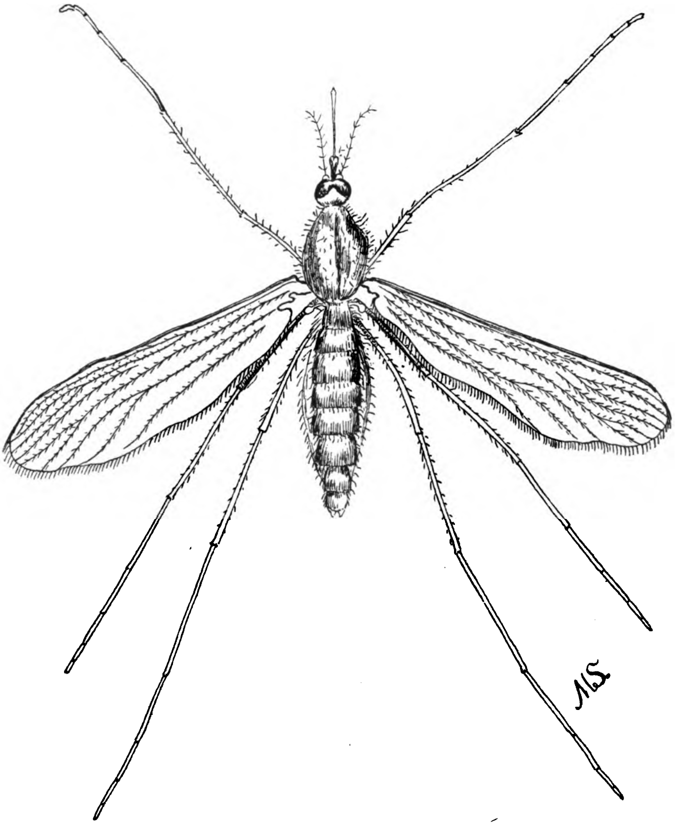

Fig. 3.—Adult female, *Culex pungens*; palpi are separate all the way to the head. Of the legs, the last five joints make the *tarsus*. The next joint is the *tibia*, the next the *femur*. The two next in order, *trochanter* and *coxa*, are short joints close to the body, and out of sight in the drawing. (Original).

Only the female sucks blood, and this is a general law for all the *Culicidæ*, with a few doubtful exceptions (Howard, 1; Ficalbi, 2). The male subsists upon the juices of plants and fruits. It sometimes eats nothing, dying soon after copulation.

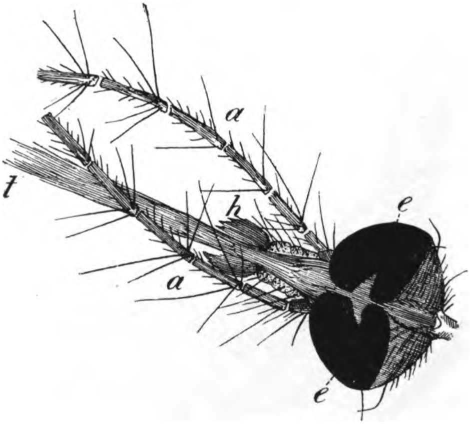

Fig. 4.—Head of female Culex (drawn by mistake from the lower instead of upper side); *e*, *e*, compound eyes; *h*, palpus; *t*, proboscis (end not drawn); *a*, *a*, antennæ (only six of the fourteen joints drawn). (Original preparation. Plate loaned by Messrs. Wm. Wood & Co.)

As to *habitat*, the genus Culex is coextensive in distribution with man and the other warm-blooded animals. Various species are found from the equator to the frigid zones. Coaches, railway carriages, winds and migrations (1,35) may bear them many miles, but by ordinary unassisted flight they are not thought to travel more than a few hundred yards from the original breeding pools.

None of the *Culicidæ* can reproduce without water. Culex breeds in natural pools of all sizes where fish are absent, in sewers, cesspools, drains, rain-filled tomato cans, rain-pools in hollow trees and post-holes, vases, fountains,—in short, anywhere where water stands for 2 or 3 weeks without drying up. Brackish water will sustain them. Sea water is not suitable for more than a few species (2,35). Hardly any degree of putrescence or contamination in fresh water seems to kill

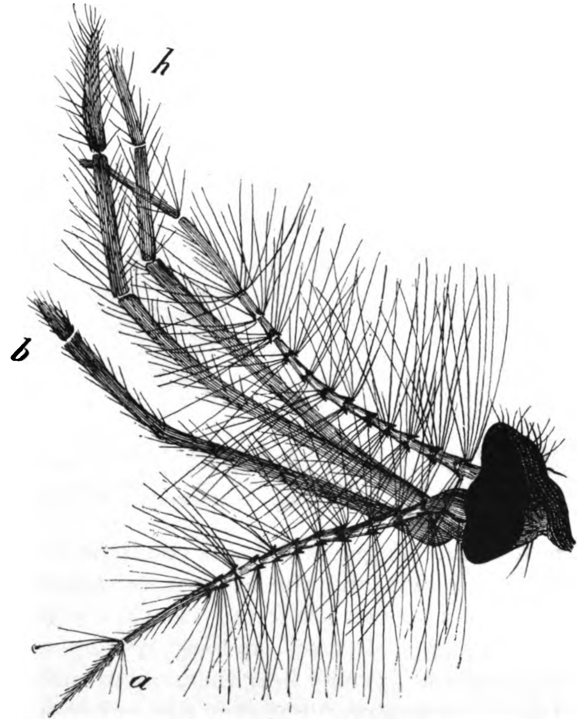

Fig. 5.—Head of male Culex (side view, head somewhat distorted); *a*, antennæ; *b*, proboscis; *h*, palpus.
(Original preparation. Plate loaned by Messrs, Wm. Wood & Co.)

them. In a brewery drain foul enough to be offensive 100 yards away I have found thousands of them.

Small fish are the greatest natural enemies of the mosquito in the aquatic stage, but they cannot be relied on to keep a pond entirely clear if marginal vegetation be abundant.

It should be noted that cold weather, rapidly cooling the breeding-pools, indefinitely retards the metamorphoses of all the *Culicidæ*,—a fact to be recalled constantly when time-limits are mentioned. Larvæ still untransformed in late autumn may be frozen in or under the ice and hibernate thus (J. B. Smith, quoted by Howard, *l. c.*), completing their development in the spring. The fully developed Culex larvæ to be found around New York early in April in pools in the woods are likely such survivors.—Fertilized adult females also hibernate in cellars, caves, stables and cold garrets, dying in the spring after oviposition. It seems well established that males do not hibernate but die early in the fall.

The **length of life** of the adult male Culex is usually only a few days; that of the females in ordinary summer weather in the open is not exactly known, but is much longer. Confined under favorable conditions they may exceptionally live 2 months or more, but usually die within 3 or 4 weeks.

ANOPHELES.

The transformations of *A. maculipennis* Meigen are described, as being most important and familiar.

The **ovum** is white when first laid but on exposure to air soon blackens. The minute structure is well described by Nuttall (3) in about these words: "The egg is boat-shaped, one side being flatter than the other. Both sides are marked with fine reticulations due to a delicate membrane which sometimes splits off. The 'rim' of the boat is ribbed, the ribs being most prominent centrally. One end of the egg is somewhat larger than the other." See Fig. 6. Of a large number of eggs laid in captivity under my own observation the number has ranged from about 25 to 75 in one lot·

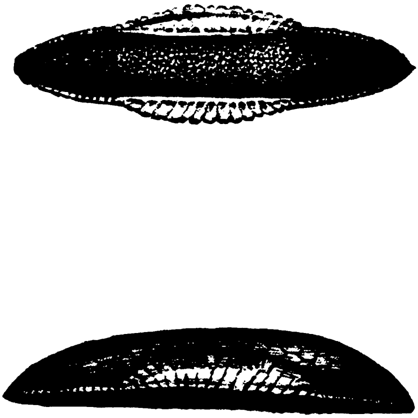

Fig. 6:—Eggs of *Anopheles maculipennis*.
(From Nuttall and Shipley).

Until agitated (when they scatter) the eggs lie flat on the water and close together, either laterally apposed or grouped in pretty triangular figures. The figures are, I think, due to the ordinary laws of adhesion and capillarity, the eggs being extruded one by one and loosely adherent to one another at the ends. Much variation in grouping has been observed and one should be careful not to generalize too freely from a few observations.

The egg hatches in from 36 to 48 hours in hot weather (88°–96° F.,34°–36° C.), the larva emerging through a circular hole (Nuttall, 3) in the larger end. It has a very short breathing tube, and unlike Culex swims and feeds on the surface of the water, deriving support not only from its air-siphon but from the racemose hairs along the sides of the body, which indent the surface-film. See Fig. 7. While small the larva can hardly be driven below the surface except by vio-

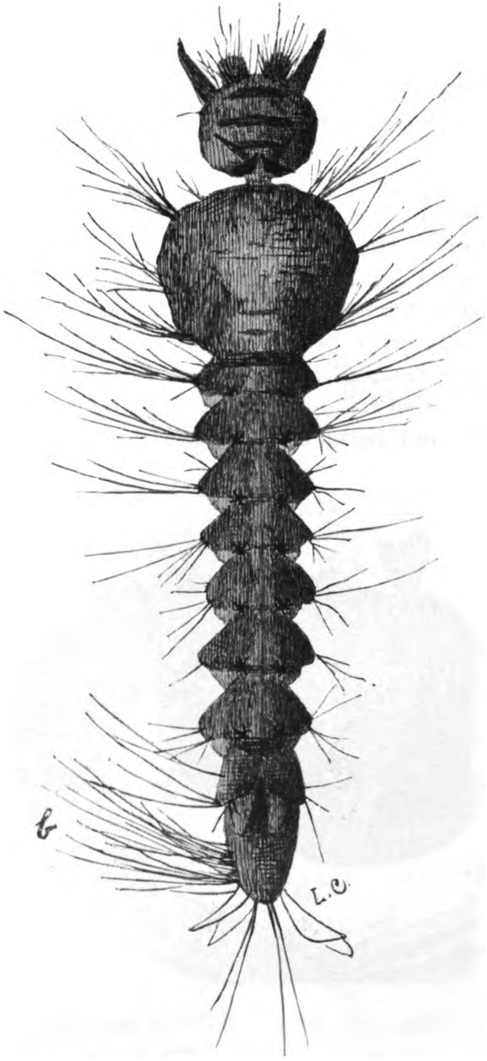

Fig: 7.—Full-grown larva, *Anopheles maculipennis*. Head in anatomical position. Breathing tube projects upward from eighth abdominal ring. Caudal brush, *b*, drawn in profile. (Original).

lence, but when half or fully grown it seeks safety indifferently either by wriggling away on the surface or sinking to the bottom. In the latter situation it may remain for 10 or 20 minutes (Howard, Nuttall), but usually returns to the surface in one-half to 2 minutes. It feeds frequently with the head rotated a full half circle on the body,—the dorsum of the thorax and abdomen and the ventral side of the head facing upward.

Smaller larvæ like to rest their caudal hairs on some support, and may be found in long rows around the sides of the dish, all facing centrally. There are frequently whitish triangular markings along the back of the thorax and belly, and the color of the entire larva

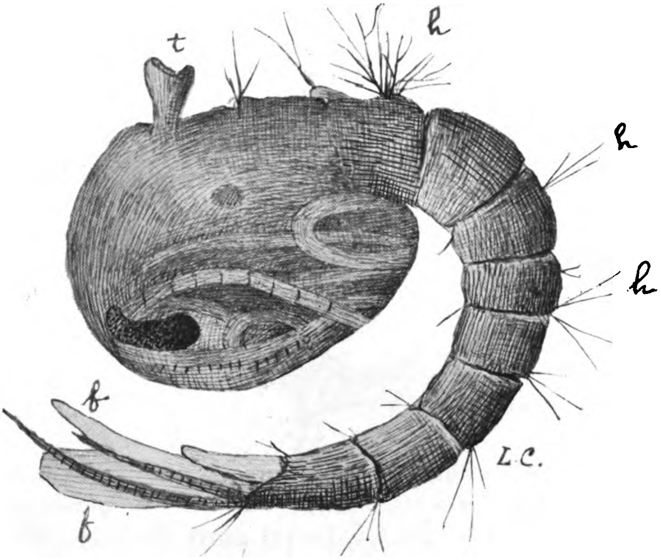

Fig. 8.—Pupa of *Anopheles maculipennis*; *t*, one of the two breathing tubes; *f*, *f*, flaps attached to last abdominal ring; *h*, *h*, hairs projecting from abdominal rings, drawn exactly as found in a newly "born" pupa. (Original).

varies much with its food. An exclusive diet of green Algæ soon turns the entire larva pale green (Howard).

The **pupa** (Fig. 8) is so much like the pupa of Culex as to make the distinction *intra vitam* rather difficult. When the larva (of Anopheles) shows the whitish markings just spoken of they will persist in the pupa. The siphons in the Anopheles pupa are nearer the middle of the thorax than in Culex and the hairs along the convex surface of the abdomen are quite different. Grassi (4) claims, quoting from G. Noè, that in life the convex profile of the abdomen in Anopheles is a per-

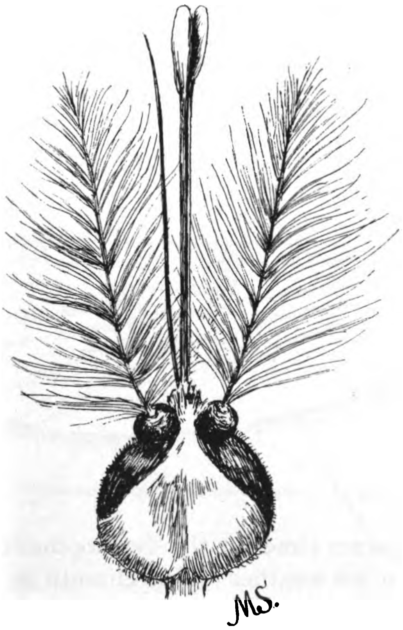

Fig. 9.—Head of *Anopheles punctipennis*, male. The clubbed palpi (to right of proboscis) are separate all the way to the head. (Original).

fect curve, whereas in Culex the joints of the rings project. I am not able fully to confirm this.

The male imago appears, as with Culex, before the female, having undoubtedly a shorter average period of metamorphosis. Both male and female are marked by *palpi as long as the proboscis*, the male palpi (Fig. 9) being clubbed. The adjacent figures (Figs. 10, 11, 12, 13) are careful studies of *A. maculipennis* Meigen, *A. punctipennis* Say (both common American species), and *A. crucians* Wied., which is much rarer. If we add to this *A. argyritarsis* (see Chapter IV), we have the only species known in the United States. Theobald (5), in his just issued monograph, has reported from the entire world about 42 species.

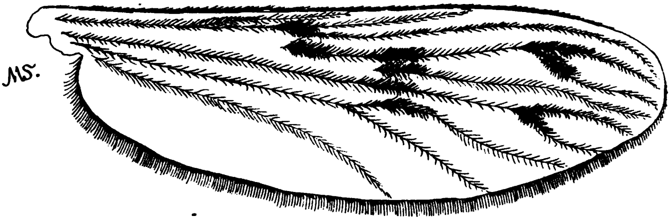

Fig. 10.—Wing of *Anopheles maculipennis*; enlarged. (Original).

The minimum time for the development of *A. maculipennis* in hot weather in this climate as observed by me in August, 1901, was exactly 15 days. Most observers (Grassi, Howard, Ficalbi, Nuttall) working in colder air or with insufficient food have reported much longer periods.

The *sitting posture* of all species of adult Anopheles, so far as known, is peculiar, and during life greatly facilitates their identification. Sitting on a perpendicular wall both sexes of all observed species incline their

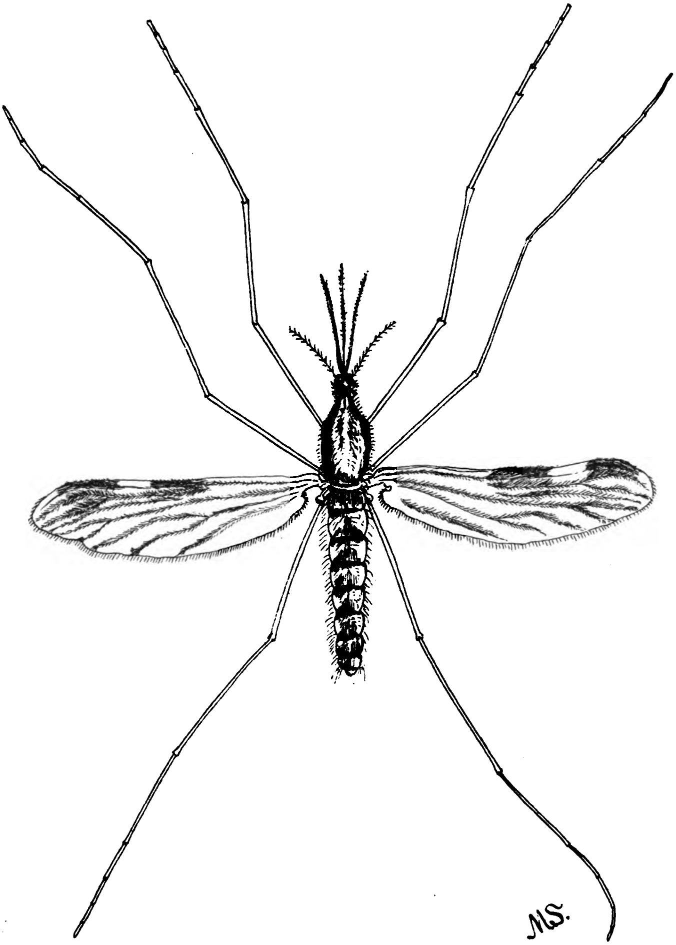

Fig. 11.—Adult female, *Anopheles punctipennis*.

bodies at an angle of from 20° to 80° with the plane of the wall. The proboscis nearly or quite touches the wall, while the last pair of legs is habitually extended

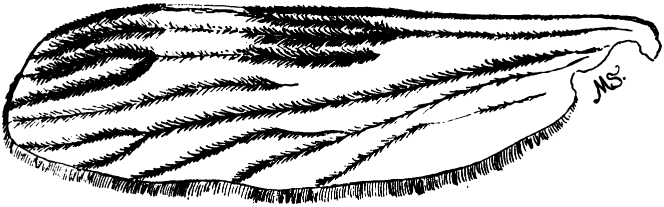

Fig. 12.—Wing of *Anopheles punctipennis*; cross-veins not drawn. (Original).

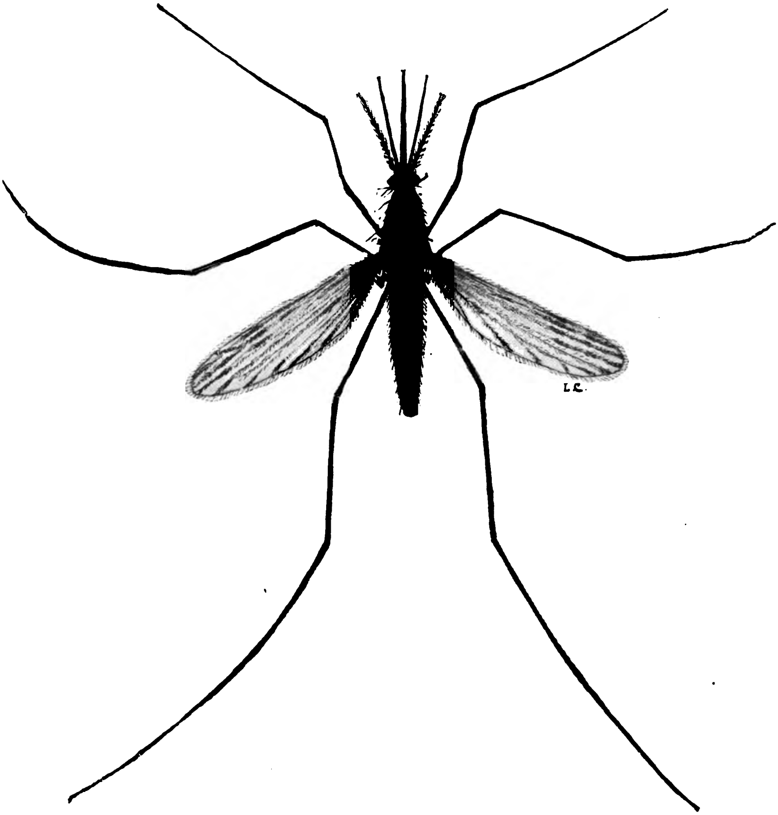

Fig. 13.—Adult female, *Anopheles crucians*. (Original).

backwards and slightly below the level of the body. Healthy and well-fed insects at large show this mark much more evidently than those in confinement. The adjacent figures (Figs. 14 to 19) are careful studies from life of *A. punctipennis* Say, and *C. pungens*, and they illustrate the postural differences perfectly. Culex hanging from a ceiling is often suggestive of Anopheles, however, and when they rest on cobwebs the difference between the two genera can hardly be made out from this mark alone.

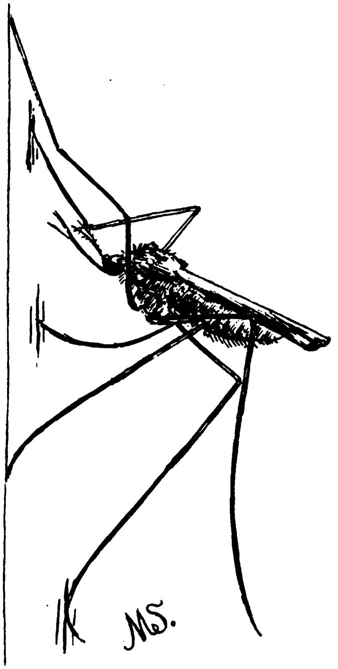

Fig. 14.—*Anopheles punctipennis*, posture on wall. (Original).

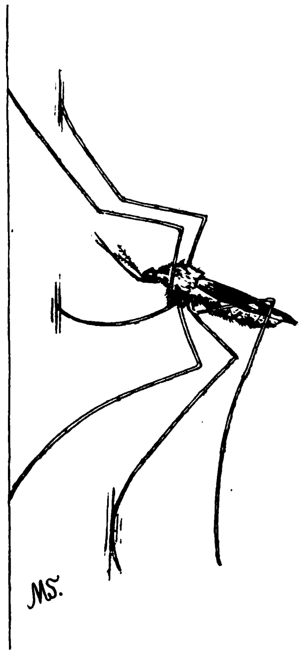

Fig. 15.—*Anopheles punctipennis*, posture on wall. (Original).

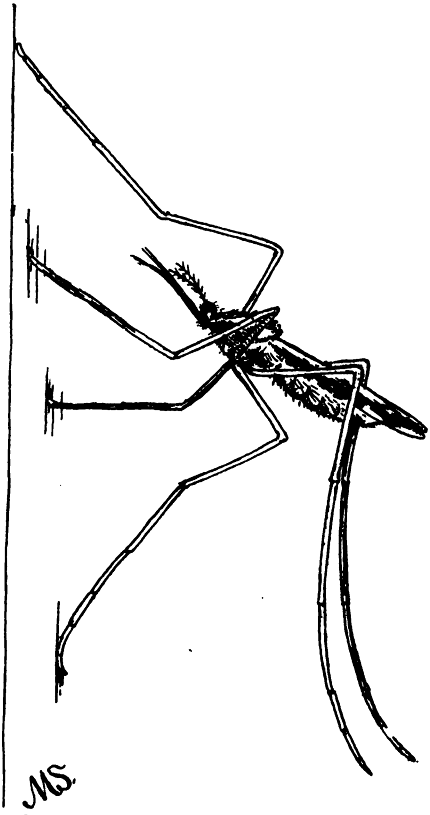

Fig. 16.—*Anopheles punctipennis*, posture on wall. (Original).

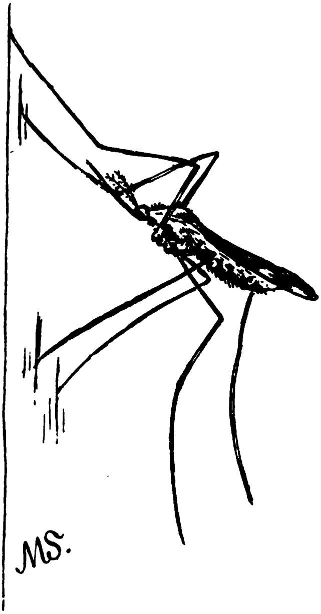

Fig. 17.—*Anopheles punctipennis*, posture on wall. (Original).

Habitat and habits. Anopheles lays by preference in natural accumulations of rain-water on the ground (Ross, 39), e.g., rain-pools, ponds, sluggish streams, surface drains, canals, pools in post-holes, small lakes where fish are absent, and reservoirs. In the absence of water of this kind they will lay anywhere where Cu-

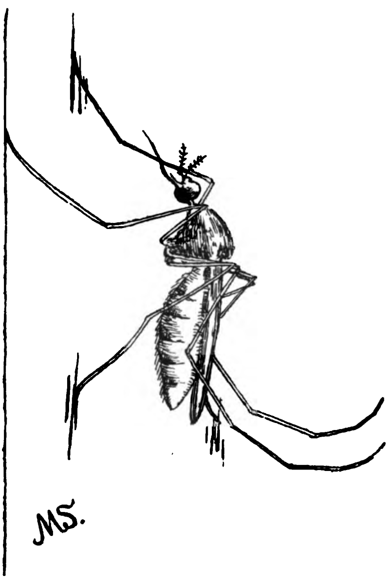

Fig. 18.—*Culex pungens*, posture on wall. (Original).

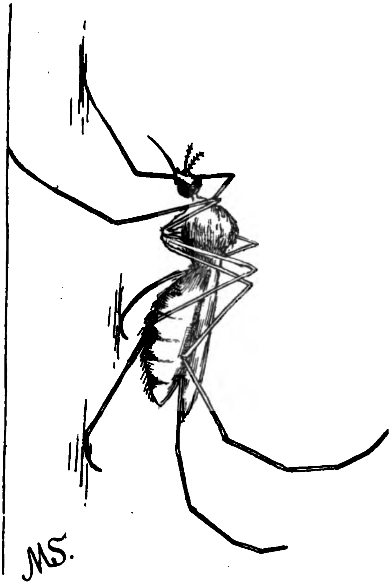

Fig. 19.—*Culex pungens*, posture on wall. (Original).

lex might, and if no water is to be had, on dry objects, e.g., the wall of a confining test-tube. Brackish water (Howard, Nuttall) and in the case of one species sea water (Bancroft, quoted in literature) will support the young. This makes Anopheles rather a suburban or rural than an urban mosquito, though this rule is not a rigid one. In New York City they may be found in Central Park in large numbers and they spread thence to widely distant parts ot the city. The adult female is said not to lay eggs till after a meal of blood. This she usually seeks from man, but bites many of the lower animals as well, biting mostly at twilight or in the night, much more rarely by day.

It is a curious fact that Anopheles one year may be abundant in a particular locality and the next year entirely absent. I have noticed this in certain parts of Bronx Borough repeatedly.

The *length of life* of the adult insect in the open is not known. In confinement in summer the male dies quickly. The female has the same average time of life as Culex. Exceptionally females may live 2 months, and of course when hibernating, longer yet; but those kept for experimental purposes in cages in the warm season will, in my own experience, rarely live over 25 days. This coincides fully with the experience of Grassi (4).

Hibernation is governed by much the same rules as with Culex, though larval hibernation has not, I believe, been so widely observed. Experimentally I have found that larvæ removed (in a small dish) from a warm room directly to the ice-box die in a few hours, but this is a very different procedure from the slow cooling of an outdoor pool in autumn.

PSOROPHORA.

This mosquito has a wide distribution in the United States but is nowhere common. Around New York I have never captured either larvæ or adults. My acquaintance with it is due entirely to the generous scientific enthusiasm of Mr. Wm. P. Seal, of Delair, N.J., who during the summer of 1901 sent me several hundred larvæ caught in pools in his own vicinity. It is marked by the erect scales on the legs (Fig. 20) and is the largest of all the American *Culicidæ*.

The eggs are large, nearly 2 mm. in length, and "prickly" (Fig. 21). Like other culicid eggs they darken in air. Those laid in captivity by my insects

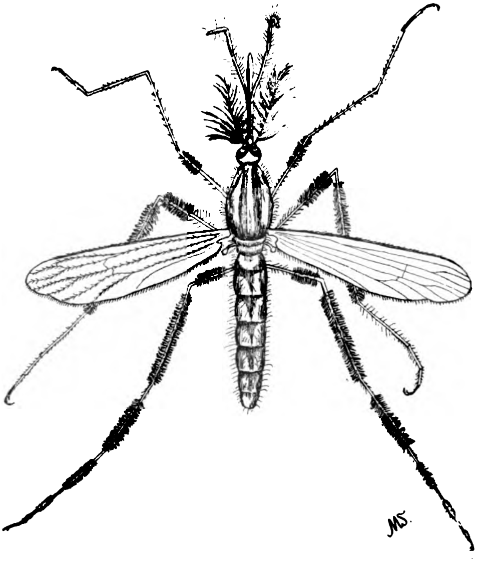

Fig. 20.—Adult male, *Psorophora ciliata ;* palpi are separate all the way to the head ; only the marginal wing-scales drawn on right side. (Original).

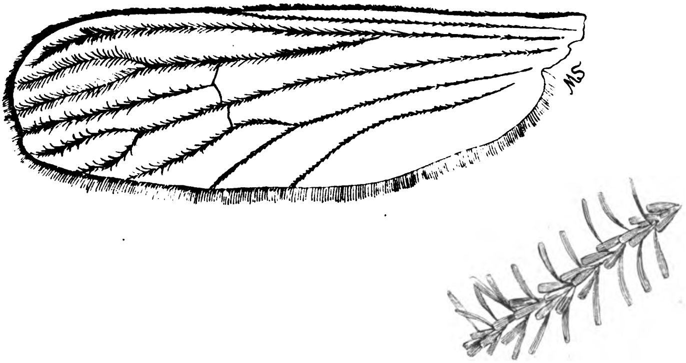

Fig. 20A.—Wing of *Psorophora ciliata*. To right, below, is drawn a segment of an internal vein, with scales, much magnified. (Original).

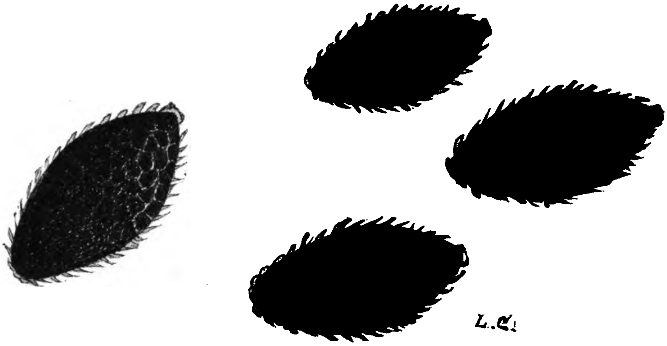

Fig. 21.—Eggs of *Psorophora ciliata.* (Original).

were grouped precisely like Anopheles' eggs and numbered as high as 75 in one lot. The ovipositing insects had been fed on blood and were 5 or more days old.

One had been in a small wire cage *without a male* since birth. Unfortunately none of the eggs hatched, so that the question of parthenogenesis remains unanswered.

The larvæ (Fig. 22) bear a strong generic likeness to Culex larvæ, but soon grow so large that they can be easily distinguished, being over ½ inch (2.5 cm.) in length when full grown. They are amazingly voracious, and eat not only vegetable food but devour Culex and Anopheles' larvæ and the weaker individuals of their own species also, being pronounced cannibals.* A large Psorophora larva may swim about for 3 or 4 hours with the remnants of a smaller one in its maw, the process of swallowing being one of slow mastication. Larvæ attack pupæ in the same way, usually eating off the tail first.

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* The allied genus *Corethra* is also cannibalistic (Giles).

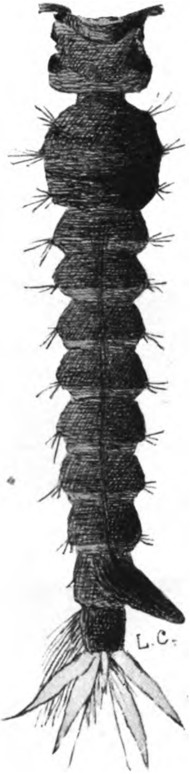

Fig. 22.—Full-grown larva of *Psorophora ciliata*.
(Original).

The **pupa** (Fig. 23) is also relatively large, and may be known easily by its size.

The **adult male** is drawn in Fig. 20. The female has pilose instead of plumose antennæ, and palpi about one-third the length of the proboscis. The posture on the wall is that of Culex.

Mr. Seal has calculated * that the entire metamorphosis in warm weather takes 10 or 12 days. The adults are of rather terrifying aspect and the females bite viciously at any time of day or night. They make large urticarial wheals which have sometimes persisted on my own hands for a week. The large size of the veneno-salivary glands (Fig. 40) may be partly the cause of this. The noise made by both males and females in flying is quite alarming,—nearly as loud as that of a bee. Women and children under such circumstances make poor subjects for an experimental bite.

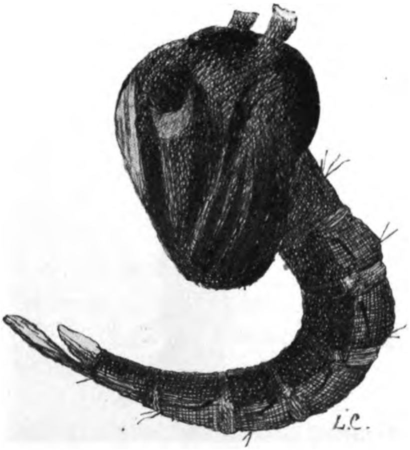

Fig. 23.—Pupa of *Psorophora ciliata*.
(Original).

The *habitat* of Psorophora is not determined. The adult has been seen in yards and gardens (Wm. P. Seal, letters; Howard) but very rarely. It is probably not a house insect. The blood the female seems to require is probably gotten from lower mammals or from birds. Dr. J.M. Callender, of Brooklyn, who saw some of my live specimens, told me he had seen the same insect while hunting in the swamps of southern Virginia. Previous to 1900 Mr. Seal, who is a naturalist of 25 years' experience, had never seen either larvæ or adults about his own house.

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* Private letter.

MEGARHINUS.

The metamorphoses of this mosquito have not been studied, so far as I know from published accounts. It is marked by a curvilinear proboscis. It has not been observed north of Washington. Southern workers have been repeatedly urged by Howard to work out the biological and pathological questions it presents. Grassi (4) remarks that it has undoubted affinities with Anopheles.

AËDES.

In the United States this is a very rare mosquito. Its biology has been recently worked out (Howard, personal communication) by Prof. Jno. B. Smith, of New Jersey, and will be published in the next edition of Howard's *Mosquitoes*.

STEGOMYIA.

See Chapter IX.

The other genera are rare, and their life-history in many important details is yet to be studied. Names and classification will be found in Chapter IV.

CHAPTER II.

COLLECTION OF MOSQUITOES. FEEDING. BREEDING.

WHERE ADULTS MAY BE FOUND.

Some general remarks on habitat have been already made. **Culex** can generally be found in the open air in summer, day or night, in regions where it exists. Some species are peculiarly abundant in woods, fields, and thickets. Those that have entered houses and sucked blood there may often be found clinging to the walls and ceilings of bedrooms, or hanging on cobwebs in dark corners of clothes-closets. After frost in autumn females are still quite abundant under viaducts, in cellars, cold garrets, caverns and stables, long after they have disappeared from the outer world. A florist at Woodside, L.I., where mosquitoes swarm like flies through the summer, told me that after the first autumn frost they could be removed from his cellar "by the shovelful." They may be found in most greenhouses the winter through,—a useful fact when a supply of specimens is needed for class demonstrations, or some other special purpose.

More experience is required to discover the adults of the genus **Anopheles.** While they are said to be found out of doors without difficulty in parts of this country and England, biting by day like Culex (Howard, Theobald), yet as a rule in the temperate zone and the tropics, they lie concealed by day in trees or shrubbery, in dwellings, or under some partial cover, and seek blood only between sunset and dawn. After 2 years' search I have never yet in the latitude of New York caught an adult Anopheles in the open air but once. In regions where they are believed to be present the most promising places to search by day are ill-ventilated bedrooms (laborers' quarters and city tenements), stables, old-fashioned box privies and the dark corners of stone archways or viaducts. They are abundant under the viaducts in Central Park. There is no doubt that they (our native species at all events) prefer as a refuge by day ill-smelling and damp, dark places, at least partly shut in from the open air.—When cold weather begins they hibernate in much the same way as Culex does. I found both genera in a dark cellar in the Bronx on November 6 and December 25, 1900, and January 6 and 13, 1901,—the weather during the last month being quite severe.

These remarks will give sufficient hints as to the places where the other genera may be looked for.

COLLECTION.—ADULTS.

The entomologist's muslin net is not desirable for mosquitoes.* Their scales are easily damaged, and legs and wings often broken in the fabric. From the wall or ceiling, or when lighting upon the clothing they are easily caught in a large-sized test-tube, which may be temporarily closed with the thumb, this being replaced by absorbent cotton.

Ficalbi (2) uses a large flask with a wide neck. A flat cork fits in the neck and a glass funnel with tube of large bore is inserted into the cork. The funnel is placed over the mosquito.—Not more than two mosquitoes should be put in one tube; they easily injure one another, the weaker ones soon dying. The tube should not be used longer than urgently necessary; blood-gorged mosquitoes soon stick themselves up in their own feces, losing legs or tarsi, particularly when changes of temperature in the room have caused dew to deposit in the tube.—A temporary cage can be easily made by fastening muslin or tacking wire-gauze over the top of a small cigar box,—leaving one corner of the cover detachable; under this the test-tube may be stuck. The mosquitoes are much less liable to do themselves harm thus, and may be transferred to permanent cages at leisure.

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* Directions for making one may be found in any of the insect-books.

Cages.—Many patterns have been devised. Howard recommends (*l.c.*) battery jars covered with "swiss." Dr. Agramonte (p. 104) uses much the same. The cage of my own devising, drawn here (Fig. 24), has a bottom and end of smooth wood and is covered with fine-mesh wire gauze, and provided at one end with a "sleeve" of muslin or linen (not mosquito-gauze, which is very insecure). The ingenious notion of a sleeve I owe to Dr. Rees's illustration in the *Practitioner* (10). My cage is perfectly ventilated, light, secure and inexpensive. A handy boy can make a half-dozen of them in an afternoon. Rubber elastic may be used to close the sleeve.

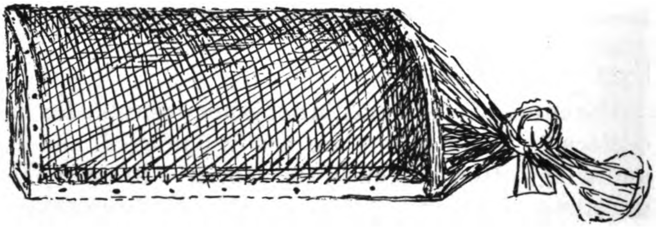

Fig. 24.—Writer's pattern of a mosquito cage.
(24 x 12 x 12 cm.).

The mosquitoes bite directly through the wire with great readiness under proper conditions, and the cage can be carried readily to the patient's bedside. The wire-mesh should be not more than 2 mm. (1/12 inch) square. The male mosquitoes particularly show a wonderful facility for creeping through small holes, and those bred in captivity are often unusually small.

This cage is specially meant for inoculation work. A breeding cage of satisfactory pattern has been described by Dr. Rees (*l.c.*). Essentially it consists of a large wooden box with wire-gauze over the front (which slides in grooves) and on the top and one end, and a large sleeve at the other end. This cage is also useful when one is working with birds. A rough one can be readily made of a soap-box. A small greenhouse is an excellent breeding place for Anopheles or Psorophora, if the doors (which should be double, with an entry between), and windows are well guarded by wire-gauze. Dragon flies, spiders and ants should be exterminated before the mosquitoes are let loose. The larger mosquitoes conjugate much more readily in large cages. Grassi (*l.c.*) used a cage large enough to hold a man and conducted some of his inoculation experiments thus. Dr. Sambon's little wire-gauze cylinders (Rees, *l.c.*) are also excellent for transporting mosquitoes.

Closed jars are not desirable. Ventilation is cut off, and the food, if left any time in the jar, is apt to grow mouldy. Mosquitoes with me die very soon after eating mouldy food. Clean, sterile rain water should be supplied to all cages in small dishes. Eggs laid in this can be transferred to other media later, while the mosquitoes run much less risk of sickness from drinking contaminated water. Sometimes with specimens one particularly wants to save from drowning (a common death when the mosquito has sickened somewhat in confinement) the water may be removed and moist sterile cotton put in, or the food renewed more frequently.

The cages should be kept clean, and in a well ventilated, half-lighted room. To keep ants away see p. 102, or smear the legs of the supporting table with rancid vaselin or ointment.

Food for Adults.—One secret for keeping mosquitoes alive for a long time is to give them only *sterile* food. One does not need much experience in dissection of mosquitoes recently dead to find out that many of them have died with the hind intestine *packed* with bacteria. This may not, of course, be the cause of death, but it seems reasonable to think so.

The best food for females is, of course, vertebrate blood. Those known not to be infected with any disease may be fed from one's hand or arm, or, in case of some genera, by putting an uninfected bird,—sparrow, pigeon, chicken,—or a rabbit into the cage. When birds are used they are best put in only at night.

Infected or possibly infected females, and all males, should be fed otherwise.—Cane sugar in 4 or 5 per cent. solution, sterilized and applied in sterile cotton to the wire of the cage may be used.* It should be removed as soon as the mosquitoes have drunk, to prevent their sticking themselves up with it and possibly loosing a leg. Feeding once in 24 hours is enough.—Fresh, over-ripe fruits are good also,—typically banana, which was first recommended by Dr. Bancroft, of Burgenpary, Queensland, some years ago. The fruit should be peeled with clean hands, sliced with a sterile knife and the slice laid on the cage-wire till the insects have eaten. Then remove it and throw it away. The fresh flowers of common red clover (*Trifolium pratense*) or orchard grass (*Dactylus glomerata*) may be put into the cage. They are not indispensable.

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*Sherry and brown sugar (equal parts) have been recommended by C. V. Creagh (5).

Though males will not suck blood I have often fed it to them from a swab, despite frequent statements that they will not eat it. As a rule, however, they should be fed upon their normal food—fruit juices. Whatever their diet, they soon die.

Most newborn females grow much darker after their first meal of blood. Some Anopheles are too pale to be identified till the wing spots have been brought out by such a meal. The same genus are said by Ross (6) not to lay eggs till they have fed upon blood. I do not think the evidence has so far established the *necessary* truth of this claim. Compare Dr. Agramonte's account of Stegomyia (p. 96).

Females in warm weather bite every 2 or 3 days. When gorged with blood or other food they never bite. Sometimes they puncture the skin without biting. Even in summer they may live a week or more without food,—only water. After frost they eat less and less and when hibernating are believed to eat nothing. In temperatures below 75° F. (24°C.) mosquitoes will rarely bite,—and the bites if gotten are negative so far as malaria and yellow fever are concerned.

Collection of Larvæ.—The differences between the breeding places of Anopheles and Culex larvæ have been already described. Those who have not seen live specimens can identify the larger ones readily from the drawings and description already given.

Nuttall and Shipley (3) think that the larva of *Dixa* may be mistaken for that of Anopheles, and they note the following differences: Dixa floats beneath the surface film (like Anopheles), but its breathing siphon is much larger and only this and the vibratile mouth-parts touch the surface, the body being concavely arched. Dixa has no palmate hairs along the body, but a whorl of simple setæ around the siphon. It swims *head first* when disturbed. Grassi (4) notes other differences.

Discovering smaller larvæ requires some experience, particularly Anopheles' larvæ, which are apt to hide in leaves and grasses at the edge of a pool and be quite invisible against the dark background of the bottom. Waiting for them to move will often disclose their hiding places; a reading glass can be used as a searcher. A large spoon or coffee-strainer and a bottle of any convenient size half full of the water they were caught in and provided with a good cork are sufficient apparatus. A beer bottle with patent stopper is useful. Three or four of these can be carried in a small hand-bag and will hold all the specimens one usually needs. The air in the bottles does not need to be renewed for hours. Rubber boots are almost indispensable. A drag-net is frequently needful. The pattern suggested here (Fig. 25) is simple and effective. For both figure and description I am much indebted to Mr. Wm. P. Seal*. The net is made of ¼ inch brass wire (unan-

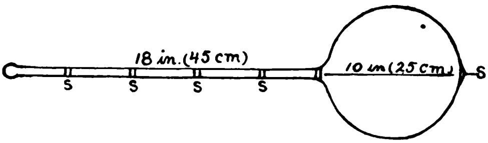

Fig. 25.—Frame for drag-net.
(Mr. Seal's pattern).

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* Personal letter.

nealed) and cheese-cloth. The wire is bent into the shape indicated in the figure and soldered at the points marked *s*, *s*. The handle is thus perfectly secure. The cheese-cloth is sewed into a bag of convenient size, the free margins of which are firmly attached to the hoop of the frame.

As before stated most small fish eat larvæ eagerly but will rarely clear a pond of them when the edges are irregular, leafy and grassy, so that dragging pays even in the most unexpected places.

Feeding and Rearing Larvæ.—Larvæ are best reared in white flat dishes two-thirds full of rain water. Into the dish should be put grass and earth from the edge of the pond the insects were caught in, and fresh water-cress may be added. Culex is very hardy and active and appears to grow readily on the microscopic flora and fauna of rain water that has been standing anywhere in the open for a few days. Anopheles feeds more exclusively upon organisms that grow upon the surface of stagnant water, such as diatoms, *Protococcus* (the familiar unicellular green alga starting up in faucet water anywhere that has stood in a dark place uncovered for a few days), *Spirogyra* and *Mougeotia* (Howard, *l.c.*). Almost any other of the fresh-water *Algæ*, except the very coarsest, seems to be suitable. Workers not familiar with cryptogamic botany may use with safety any of the excessively fine, impalpable green filaments growing familiarly in horse-troughs, ditches and frog-ponds everywhere in the temperate zones. Larvæ that die are usually underfed. Several authorities (Grassi, *l.c.*, and others) remark that a second lot of larvæ do ill in water previously used for a first lot. I have not found it so when enough food was supplied. The bodies of dead adult mosquitoes make a good food; they are soon gutted by the larvæ. The tops of the dishes should be covered with thin muslin to exclude dust, particularly the dust of laboratories, which is apt to be poisonous. Half-light is best; I have seen pupæ die quickly when exposed to direct sunlight. As soon as pupation begins the dish should be put inside of a cage. Raw rice in the breeding dishes has been recommended by Dr. Rees (*l.c.*).

More than two-thirds of the young should be matured if sufficient care has been exercised; if not, deaths will be frequent, and hardly half will mature.—A word should be added as to Psorophora. The larvæ have been found, so far as my information goes, only in natural pools. They may be secured by dragging. They are violent cannibals and should be kept in cups or small dishes isolated from one another. They feed well on the diet already described and may also be fed on other culicid larvæ, which they will attack, even when vegetable food is plentiful.

Collection of Eggs.—The little "boats" of Culex eggs may be found in the open by those who know what to look for. Anopheles eggs, scattering very soon, can rarely be found except when laid in confinement. Well-developed, blood-gorged females of almost any of the genera caught in bed-rooms and kept at 80° to 90° F. in a cage with a dish of water will usually lay on the first night after their capture. Being almost always fertilized when caught the insects will drop a second lot of eggs and even a third if blood be further supplied in large enough amount. In summer laboratory-bred mosquitoes will drop eggs a few days after hatching if males are present in the cages, if the cages are large enough to permit copulation, and if blood be fed the females. The temperature should be over 80° F.

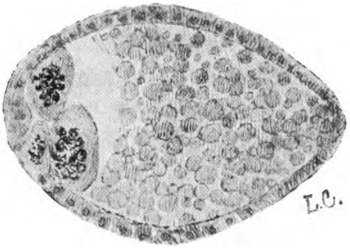

Fig. 26.—Mitotic nucleus, egg of Anopheles *in utero*.
(Original).

Healthier insects can usually be gotten from larvæ caught out of doors; and it should be remembered that experimentation with any but healthy insects is not only useless but may be gravely misleading.* Mosquitoes frequently drown while or after ovipositing. Eggs laid on a dry surface remain viable for a time which varies with the different species. Ross subsequently questioned a former statement of his, to the effect that the eggs of Anopheles may remain viable for six months when kept dry (Nuttall, *l.c.*). See, however, Dr. Agramonte's remark (p. 98).

An Anopheles (*A. maculipennis* Meigen) in my laboratory kept in a test-tube over night on one occasion laid a large lot of eggs *which remained* white, never turning dark and never hatching.

It is a pretty well attested fact for all the genera but Stegomyia (p. 101) that of a given lot of eggs reared artificially about two-thirds will usually turn out males, one-third females. It is perhaps not premature to refer in this connection to the investigations of P. Geddes (7), viz., that abundant supplies of food largely increase the number of females coming from a batch of insect eggs of almost any genus. My own experiments confirm the observation entirely in respect to Anopheles. Latterly I have supplied the dishes from the egg stage on with a large excess of food and have succeeded, as a rule, in getting about twice as many females as formerly,—a great convenience, since only females admit of experimental work. I would not however, speak of this difficult and involved subject as thus easily settled.

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* The eggs when laid are in various stages of intrauterine development. Fig. 26 shows the second mitosis of the first *two* cells in an Anopheles egg *in utero*. This fascinating subject cannot be discussed here.

CHAPTER III.

PRACTICAL OUTLINES OF ANATOMY.

The limits of this book admit only of only an outline of the anatomy of the mosquito. A host of interesting anatomical details,—some of them still in dispute,—are regretfully passed in silence. It has finally seemed best, also, for brevity's sake, to omit entirely the minute anatomy of the larva and pupa. The works of Hurst (8) for the pupa of Culex, and of Giles (9) may be referred to by those interested. When one has learned how to work with adults one can soon find his own way with the larva and pupa, if further research should point in their direction.

The **Culicidæ** or **Mosquitoes*** belong to the Class *Insecta* and Order of two-winged flies or *Diptera*. In brief, they are marked off from kindred families of the same order by the distribution of their wing-scales, which are attached in fringes not only to the margins

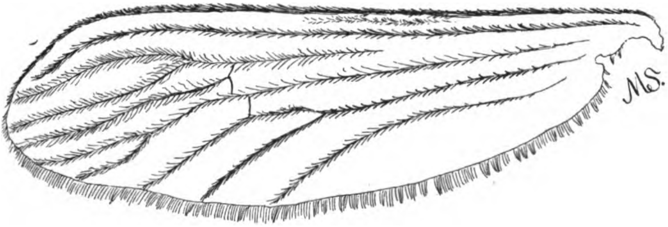

Fig. 27.—Wing of *Culex pungens* showing veins and scales. (Original).

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* This word is used in this book as the English synonym for the Latin name of the family.

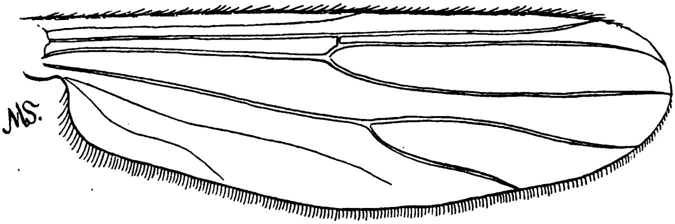

Fig. 28.—Wing of *Chironomus plumosus*, enlarged. (Original).

but also to the *veins* of the wings. A wing of Culex is drawn in Fig. 27. Beside it (Fig. 28) is a wing of Chironomus (*C. plumosus*), belonging to the allied family *Chironomidæ.* The different distribution of the scales will be seen at once.

GROSS ANATOMY.

The mosquito, like insects in general, is divided for purposes of description into a **head, thorax** and **abdomen.**

The **head** bears two large *compound eyes* (Fig. 4, *e*, *e*, p. 5), two *antennæ* (*a*), which are plumose in the male and pilose in the female (Figs. 4, 5); two *palpi*, which are of variable length and appearance in the different sexes and genera; and a *proboscis*, which though a compound organ is practically a hollow tube through which the insect feeds. Descriptions and drawings of the proboscis are numerous since Dimmock's work (11) first appeared. The proboscis has for its largest member a grooved lower lip or *labium.*

Of the **thorax** the ventral side shows six legs. The first pair is the shortest, the last the longest of the three. All bear scales of varying colors which to the naked eye determine the colors of the various joints.

The leg consists of a *coxa* (Fig. 3), *trochanter*, *femur*, *tibia* and *tarsus*. The tarsus has five joints. The last or *ungual* joint of the tarsus is tipped by claws, usually two in number and often dentated (particularly the claws of the front legs). These dentations vary much in the sexes and species and bear vitally upon the classification. Figs. 29 to 33 are careful drawings of the front claws of *Culex pungens* Wied., male and female. The same drawings show the way in which the leg-scales are attached. The claws cannot be seen without

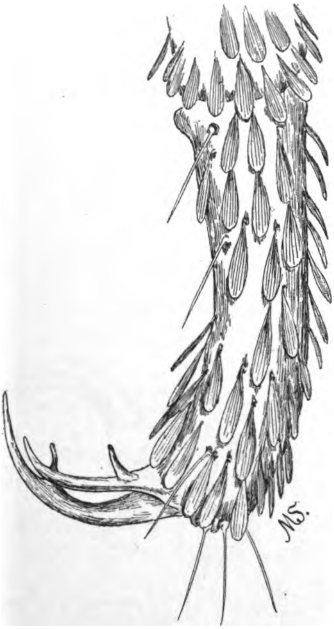

Fig. 29.—Last tarsal joint and claws, foreleg of *C. pungens*, male. Some of the scales and hairs absent. (Original).

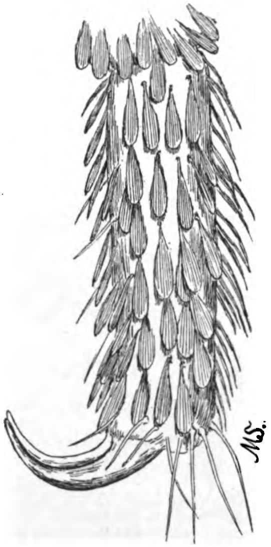

Fig. 30.—Last tarsal joint and claws, foreleg of *C. pungens*, female. (Original).

a microscope. If experienced in handling a triplet lens one can distinguish the claws thus (Howard, *l.c.*). If not, the best plan is to mount the leg or tarsus directly (dehydration not needed) in balsam-xylol under a cover-glass and examine with the low power of the compound microscope. The proper mode of studying pinned specimens will be spoken of in Chapter IV.

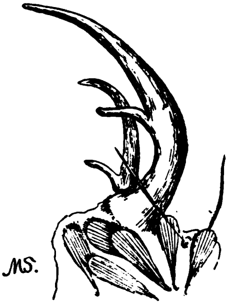

Fig. 31.—Claws, foreleg of *C. pungens*, male.

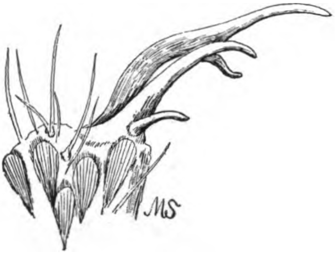

Fig. 32.—Claws, foreleg of *C. pungens*, male.

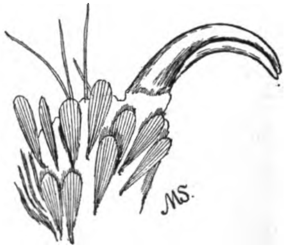

Fig. 33.—Claws, foreleg of *C. pungens*, female.

The *dorsal* surface of the thorax (sometimes divided from before back into *pronotum*, *mesonotum* and *metanotum*) presents posteriorly two minute knobs (rudimentary wings) called *halteres*, or "balancers." In front of the halteres are the true wings. These are fringed with a triple row of scales, and each vein, as just remarked, also bears scales.

The various "markings" on the wings are due only to the color and distribution of the scales. The *venation* of the wings is very constant in the several species and is a useful mark. The areas between veins are called *cells*. Many conflicting systems of naming these veins and cells have been published. Ficalbi (2) and other writers have recently abandoned these names and now use Roman numerals to mark the cells and Arabic to mark the veins,—a change which will, it is to be hoped, be generally adopted. I give the former names so far as they appear in the Key. The *costa* is the anterior margin of the wing (Fig. 34). The *first submarginal cell* is cell iv in the figure. The *petiole* of any cell is the vein running from its internal angle inwards to the next adjacent joint. The petiole of the first submarginal cell is just under the numeral iii in the figure.

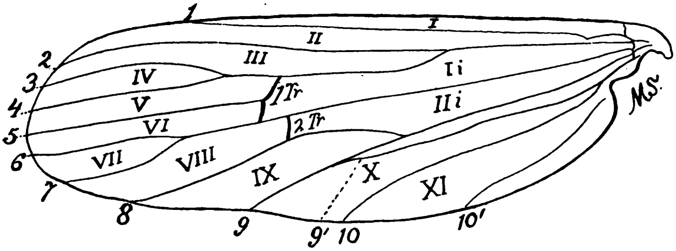

Fig. 34.—Wing of *Culex pungens*, denuded of scales to show venation. Roman numerals mark the cells, Arabic the veins. The conventional terminology of veins and cells is thus restated by Theobald:—Anterior margin of wing is *costal vein*; 1, *subcostal vein*; 2, *first longitudinal vein*; 3, 4, *second long. vein*; 5, *third long. vein*; 6, 7, *fourth long. vein*; 8, 9, *fifth long. vein*; 10, *sixth long. vein*; 1Tr., *supernumerary and mid-cross veins*; 2Tr., *posterior cross-vein*; 9′ and 10′, incrassations.—I, *costal cell*; II, *subcostal cell*; III, *marginal cell*; IV, *first submarginal cell*; V, *second submarginal cell*; VI, *first posterior cell*; VII, *second posterior cell*; VIII, *third posterior cell*; IX, *anal cell*; X, *auxiliary cell*; XI, spurious cell; I*i*, and II*i*, *first and second basal cells*. (Original).

The **abdomen** consists of eight chitinous rings or somites marked with hairs or scales of varying colors. The last segment shows the external genital apparatus, varying markedly in males and females and in the various species. Ficalbi's work may be consulted for an elaborate description.

The *special senses* of mosquitoes are at least five, and several of these are very highly developed, particularly that of smell.

The Key to the classification is assigned a separate chapter (Chapter IV).

INTERNAL ANATOMY.

The **respiratory system**, like that of insects generally, consists of an elaborate set of *tracheæ* or air tubes, with capillary ramifications so minute as to be visible sometimes only with the oil immersion lens. Every organ has its own set of air tubes; the drawing of the rectal glands (Fig. 37, p. 45), or the ovaries (Fig. 36) may be noted as an example.

Circulation of the fluid in the extraintestinal body cavity or coelom is secured by a longitudinal dorsal vessel, which pulsates somewhat like the heart of higher animals, and has "pericardial" spaces to either side of it. The "blood" of the mosquito is a colorless fluid containing no oxygen-carrying cells at all. In dissection it is usually turbid with fat droplets.

Far the most important organs for our purpose are the *alimentary canal* and *its appendages*.

The **alimentary canal** extends from the base of the proboscis to the anus, and is divided into *anterior*, *middle* and *posterior intestine*, or gullet, stomach and gut. Its appendages are the *salivary glands*, the *suctorial vesicles*, the *Malpighian tubes* and the *rectal glands*.

The **gullet or œsophagus** extends from the base of the proboscis nearly half way through the thorax to the anterior end of the *stomach*. At its beginning is affixed the common duct of the *salivary glands*; at its rear end are attached a principal and two accessory *aspiratory* or *suctorial vesicles** (drawn in Fig. 35). The largest of these vesicles extends backwards into the front of the abdomen. All three are usually full of minute air bubbles, some of which are drawn in the figure. Around the air-blebs, in carefully made fresh dissec-

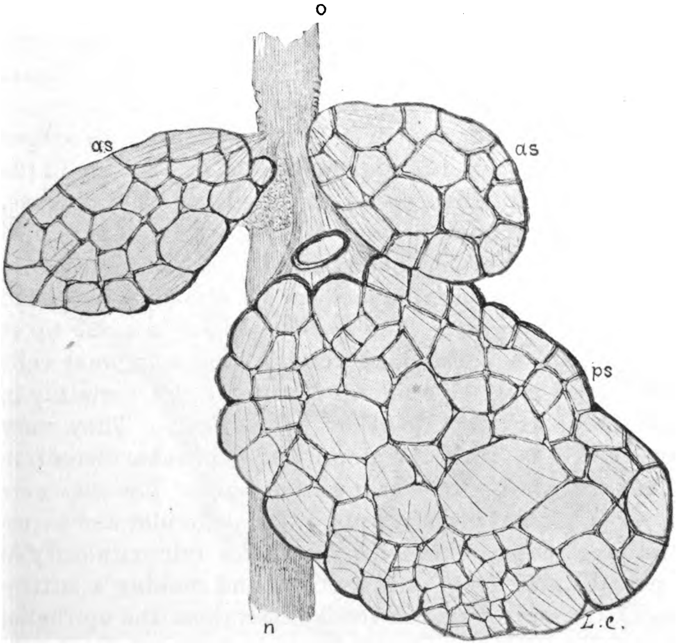

Fig. 35.—Suctorial vesicles, *C. pungens; as, as,* accessory vesicles; *ps,* principal vesicle; *n,* neck of stomach; *o,* posterior section of esophagus. The 3 sacs join the alimentary canal just in front of a slightly thickened muscular ring (the "cardiac" valve) under which large epithelia are visible. All of the sacs are full of minute air-bubbles. The wall of the sacs is a transparent membrane through which minute muscular fibrils course in various directions. (Original).

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* The figure extensively quoted from Macloskie (12) contains a serious error here, and should not be trusted.

tions in salt solution, the sacs may be seen to contract and relax violently at intervals of a few seconds. Giles (9), who describes only the largest vesicle, suggests that it may be a part of the respiratory system, as he was never able to press the air-bubbles from it into the gullet. Grassi (*l.c.*), however, thinks that he has seen blood in the sac of Anopheles after biting,—an observation which I can confirm. The outside of the valve connecting gullet and middle intestine shows little knobs.* This valve usually lies about on the level of the second pair of legs (Grassi).

The **stomach** ("ventricle," "chyle-stomach") consists (Fig. 36) of an anterior narrow "neck" originating in the thorax, as explained above, and a posterior dilated sac. The latter extends when empty from about the third to the sixth or seventh abdominal ring. The walls consist of two essential layers, an internal *epithelial* and external *muscular*. The *epithelial layer* is made up of large and medium-sized roundish or polygonal cells with well-marked nuclei. The cells are certainly in some regions "prickle-cells" (*Riffzellen*). They vary in thickness with the degree of stomachic distention but seem always to be in multiple layers. The *muscular layer* (Fig. 36 A) is made up of internal circular and external longitudinal fibres, disposed with fair regularity at some distance from one another, and making a lattice-work through which in fresh dissections the epithelial layer often bulges like baled cotton between the bands of the bale. The fibres are very tenuous, but in fresh dissections are actively contractile for a quarter of an hour or more, if the cover-glass be left off. Peristaltic waves starting in the neck of the stomach may be often seen (under the low power of the microscope) to travel all the way through the hind-gut to the anus and even part of the way back.—Exteriorly the stomach is supported by a network of air-tubes, fine connective tissue and fat.

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* These knobs in Anopheles are described by Grassi as minute blind sacs or *coeca;* in Culex they have often struck me as only localized muscular contractions, and are quite often not to be seen in the fresh dissection.

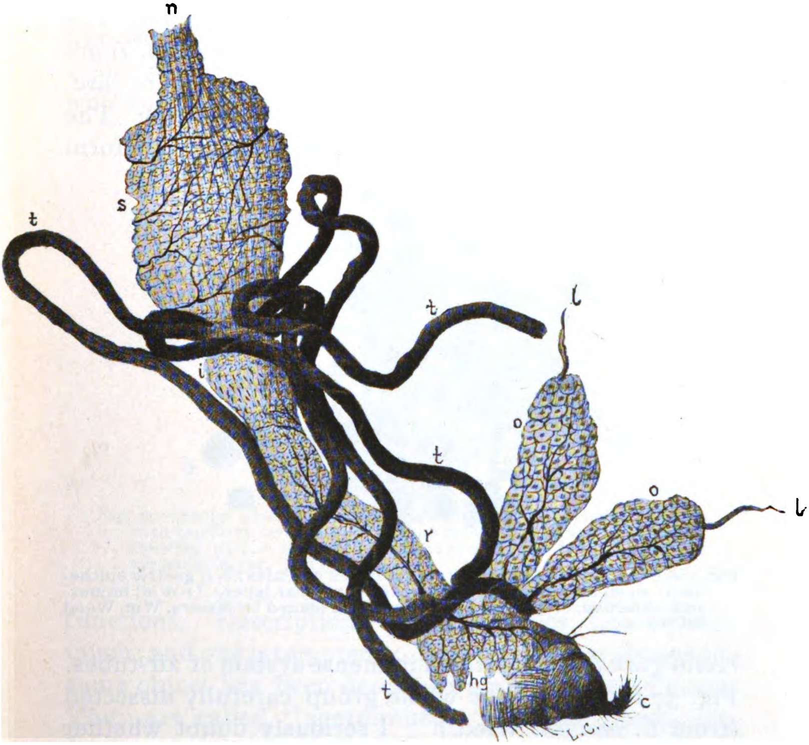

Fig. 36.—Fresh dissection, middle and hind intestine of *Anopheles punctipennis; n*, neck of stomach; *s*, stomach proper; *i*, ileum; *r*, rectum; *hg*, rectal glands (4 in sight); *t*, *t*, Malpighian tubules; *o*, *o*, ovaries, with ligaments, *l*, *l*, at anterior ends; *c*, chitinous tip of last abdominal ring. The black tracery of the air-tubes and capillaries everywhere visible. (Original).

The **Malpighian tubules** (Fig. 36, *t*, *t*) are 5 in number, and are usually disposed as in the figure (with many minor variations). The drawing is description enough. Their function is supposed to be urinary.

The **posterior intestine** or "hind-gut" may be divided into *ileum* (or *duodenum*), *colon* and *rectum*. The structure and markings of the *ileum* (Fig. 36, *i*) are peculiar. The muscular and epithelial layers are, roughly speaking, similar to those of the stomach. The *rectum* is provided with six large and stout pyriform *rectal glands*, each with an immense system of air-tubes. Fig. 37 represents the whole group carefully dissected (from *C. pungens* Wied.). I seriously doubt whether the function of these immense glands placed thus at the very end of the alimentary canal is digestive only. Nor, if they "furnish additional mucus for the eggs," should they be as large in Anopheles as in Culex.

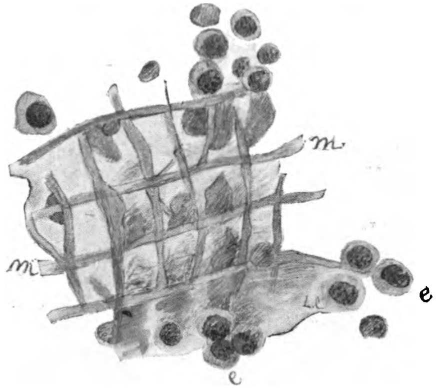

Fig. 36A.—Oblique section of normal stomach of Culex; *e*, *e*, gastric epithelium; *m*, *m*, meshwork of fibers in the muscular layer. (1-16 oil immersion objective; original preparation. Plate loaned by Messrs. Wm. Wood & Co.)

The **reproductive organs** do not specially concern us. The female has two ovaries (Fig. 36), varying enormously in size, according as the insect is gravid or not. The ovarian ducts unite at their rear ends to make an oviduct. Anteriorly each ovary is supported by a stout "ligament" (Fig. 36 *l*, *l*), which is contractile in the fresh state.

Connecting with the oviduct by pedicles on either side are 3 minute bodies, 6 in all (not shown in the figure), which are brown by transmitted light and to which are assigned by various writers a variety of functions. Descriptions of them (Giles, Grassi) vary much; and one is tempted to believe that not always the same object has been described under the same name. They are called "spermothecæ," mucous glands, etc. In Anopheles Grassi (*l.c.*) describes an additional muciferous gland connected with the ovaries.

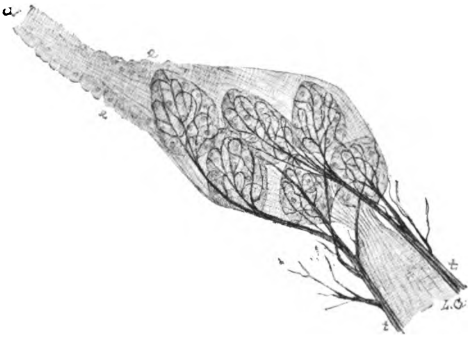

Fig. 37.—Rectal glands of *Culex pungens* (Leitz, ⅔ rds); *t*, *t*, tracheæ with capillary twigs supplying the cells; *a*, anal end of intestine showing at *e*, *e*, the large intestinal epithelia lying under the muscular layer. (Original).

The nervous system is beyond the scope of our inquiry. It may be noted, however, that all the abdominal viscera are supplied from chains of large, finely granular, nucleated cells connected by slender nerve fibers and giving off dentritic twigs laterally. A part of one chain is drawn in Fig. 38.

The **salivary glands,** 2 in number, are important organs. They lie at the base of the thorax just above the first pair of legs and below and to either side of the gullet. Each gland consists of 3 (in Psorophora 5) lobules (Figs. 39, 40). The lateral lobes are slender, longer than the middle lobe, rather sinuous, and sometimes showing small diverticula. The plane of the 3 lobes is more nearly vertical than horizontal (Grassi). Each lobe has a duct and minute companion air-tube.* The ducts of the lobules unite as in the figure, and the common duct, uniting with its fellow of the other side in the neck, finally connects with the lower side of the gullet immediately at the base of the proboscis.

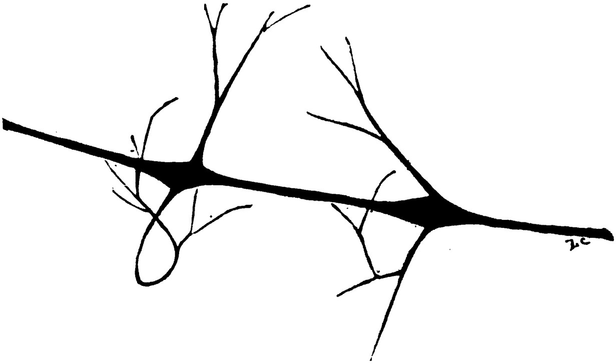

Fig. 38.—Segment of abdominal (ovarian) nerve-chain, *Culex pungens.* (Original).

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*Care should be taken not to mistake the air-tube for the duct, as certain writers appear to have done. The duct itself strongly resembles an air-tube.

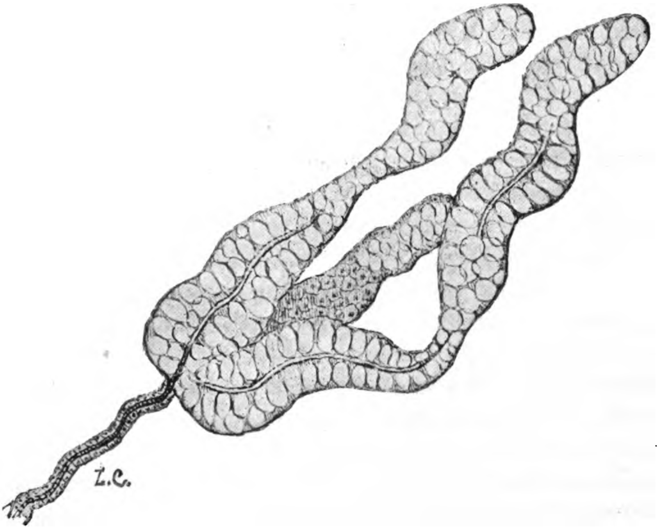

Fig. 39.—Salivary gland and duct of *Culex pungens*. (Original).

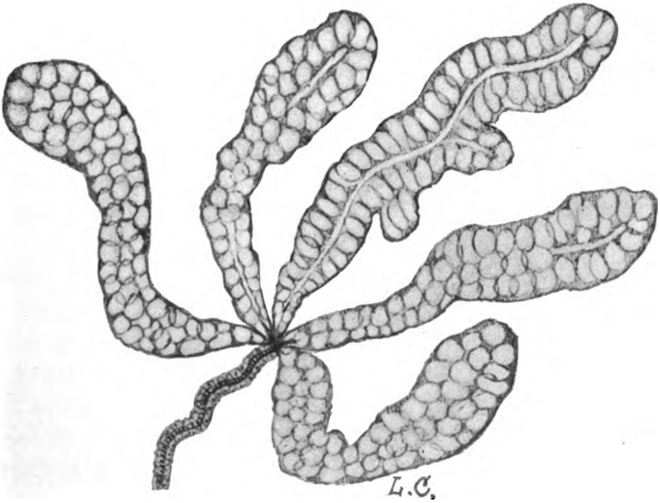

Fig. 40.—Salivary gland of *Psorophora ciliata*, fresh dissection. Middle lobe shows 2 diverticula. (Original).

The upper and lower lobes are similar in cellular structure and are a little less than 1 mm. in length (about 2 mm. in Psorophora). The cells are plump, nucleated, closely apposed, and lie in a single layer upon a hyaline basement membrane. They enclose a lumen diagramatically shown in the figure. The lumen usually contains fluid. At the bases of the cells, next the basement membrane, some smaller cells can be made out in a good fresh dissection. These possibly constitute a germinal layer, though the literature contains no comment on them that I have seen. The middle lobe is much shorter, pyriform in shape, with a larger lumen and cells of somewhat different appearance. See figures. It has been supposed that this lobe is poisonous, whereas the lateral lobes are salivary. Malarial sporozoites appear in all 3 (Grassi), though Ross and other writers describe the middle lobe as most abundantly infected.

CHAPTER IV.

KEY TO NORTH AMERICAN (U.S.) GENERA AND SPECIES.

MR. THEOBALD'S WORK.

The Classification of the Culicidæ of the world is a technical subject which is still incomplete. For a long time to come it must in its more difficult phases be left to the professional dipterologists. There is, however, urgent need that physicians (to whom this little book is specially addressed) should be able to identify readily the more important genera and species of the family as it now stands. After mastery of the first difficulties the study of the subject will prove immensely fruitful, as has been already admirably demonstrated by British physicians in all parts of the world.

The following **Synopsis of the Genera and Species of the United States,** prepared by Mr. D. W. Coquillett of the U. S. Department of Agriculture for Dr. L. O. Howard's recent work, *Mosquitoes*, is therefore reproduced here in the belief that it will form the best possible introduction to the larger and more difficult world-field. My acknowledgments have been already made to the gentlemen named (see Preface) for their kind permission to use this Table.

Explanation of the technical terms that occur may be found in Chapter III. If pinned specimens from a collection are to be studied, they may be stuck into a bit of cardboard and the whole mosquito placed under the ⅔ dry objective. Scale-structure cannot be well studied in any other way (Theobald). Wings may be mounted in balsam, or placed dry under a cover-glass.

In laboratory work with new mosquitoes at least 6 males and 6 females of the species under observation should be preserved[*] (by pinning or otherwise), that there may be no subsequent question as to the name. If pinning be elected the specimen should be pinned through the thorax in three different ways so as to show the dorsal, ventral and pleural aspects of the insect (Theobald).

I.—GENERIC SYNOPSIS.[†]

The following table contains all the genera of the long-beaked mosquitoes known to occur in North America. The males are readily recognized by the antennæ being densely covered with long hairs; in the females the hairs of the antennæ are short and very sparse:

1. Palpi in the male at least nearly as long as the proboscis; in the female less than one-half as long................ 3
Palpi in both sexes at least almost as long as the proboscis. 2
Palpi in both sexes less than one-half as long as the proboscis.. 7

2. Proboscis straight or nearly so, colors of body brown and yellowish......................................*Anopheles*
Proboscis very strongly curved, colors bluish or greenish. *Megarhinus*

3. Legs bearing many nearly erect scales.........*Psorophora*
Legs destitute of such scales............................. 4

4. Colors black, brown and yellowish, proboscis almost straight.. 5

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[*] Excellent directions for pinning and mounting insects may be found in many of the popular insect-books. Howard's instructions (1) are perfectly satisfactory.

[†] Mr. Coquillett advises me that the genus *Toxorhynchites* (see p. 56), originally included in this *Synopsis*, should be dropped. This is virtually the only change from the original except the omission of one paragraph on the unrecognized species of Culex.

5. "Thorax marked with lines of silvery scales" (Theobald). *Stegomyia*
Thorax not marked in this way.... 6

6. "Hind feet black, their apices snow-white" (Theobald)... *Conchyliastes*
Hind feet not marked like this........................*Culex*

7. Upper side of thorax with line of bluish scales.. *Uranotænia*
Upper side of thorax not marked in this way.........*Aëdes*

II.—GENUS URANOTÆNIA.

Our single species is rarely met with; it is among the smallest of mosquitoes and will readily be recognized by the stripe of violet-blue scales on the thorax.

sapphirina O. S.

III.—GENUS AËDES.

The single species is likewise rarely met with and, like the preceding, is of very small size; it is of a brownish color with golden yellow scales on the thorax and cross-bands of white ones on the abdomen.

fuscus O. S.

IV.—GENUS CONCHYLIASTES.

Our two species are of medium size and of rather rare occurrence; they may be distinguished as follows:

With the last two joints of the hind feet white.....*musicus* Say.
With only the last joint of the hind feet white..*posticatus* Wied.

V—GENUS STEGOMYIA.

Our two species are of rather small size, and may be distinguished by the following characters:

Front claws of the female toothed on the under side, the outer silvery stripes of the thorax greatly widened in front of the wings...*fasciata* Fabr.
Front claws not toothed, the outer silvery stripes of the thorax very narrow throughout their entire course ....*signifera* Coq.

fasciata Fabr.
  excitans Walk.
  frater Desv.
  mosquito Desv.
  tæniatus Wied.
signifera Coq.

IV.—GENUS CULEX.

RECOGNIZED SPECIES.

Males.

1. Front tarsal claws bearing a distinct tooth near the middle of the under side of each............................ 3
Front tarsal claws bearing two teeth on the under side of one claw, and one on under side of the other, proboscis destitute of a whitish band near the middle............ 2

2. Feet distinctly white at bases of the joints.*sollicitans* Walk.
Feet not white at bases of the joints......*consobrinus* Desv.

3. Proboscis destitute of a whitish ring near the middle..... 4
Proboscis with such a ring, ends of tarsal joints white.... *tarsalis* Coq.

4. Bases of tarsal joints not white.......................... 5
Bases of tarsal joints white................*stimulans* Walk.

5. Palpi not dilated.. 6
Palpi strongly dilated toward the apex.......*impiger* Walk,

6. Petiole of first submarginal cell at most one-tenth the length of that cell........................*pipiens* Linn.
Petiole at least almost half as long as the cell........... *pungens* Wied.

Females.

1. Front tarsal claws bearing a distinct tooth near middle of under side of each...................................... 2
Front tarsal claws destitute of teeth..................... 7

2. Proboscis destitute of a white ring near the middle........ 4
Proboscis marked with such a ring, bases of tarsal joints white.. 3

3. With a stripe of yellowish scales in middle of the abdomen.................................*sollicitans* Walk.
Without such a stripe.................*tæniorhynchus* Wied.

4. Bases of tarsal joints distinctly white......*stimulans* Walk.
Bases of tarsal joints never white........................ 5

5. Abdomen marked with a cross-band of whitish scales at base of each segment......................*impiger* Walk.

6. Abdomen never marked in this manner, but with a cluster of whitish scales at front angles of some of the segments .... *triseriatus* Say.

7. Proboscis marked with a distinct whitish ring near the middle, feet white at sutures of the joints.............. 8
Proboscis destitute of a whitish ring near the middle..... 9

8. Tarsal joints white at bases only.........*perturbans* Walk.
Tarsal joints white at both ends...............*tarsalis* Coq.

9. Feet white at bases of joints..............*excrucians* Walk.
Feet never white at bases of the joints..................10

10. Petiole of first submarginal cell about one-eighth of the length of that cell......... ....*pipiens* Linn.
Petiole one-fourth the length of that cell ....*pungens* Wied.
Petiole at least almost half the length of that cell.........
consobrinus Desv.

VII.—GENUS PSOROPHORA.

Our single species is of a yellowish color, usually varied with brown, the bases of the tarsal joints white. It is considerably larger than any of our other species of yellowish or brown mosquitoes:

ciliata Fabr.
molesta Wied.
? rubida Desv.

VIII.—GENUS MEGARHINUS.

Our three species are among the largest in this family, and are not known to occur north of the District of Columbia. They may be separated as follows:

1. Hind feet alone marked with white......................... 2
None of the feet marked with white .. *hæmorrhoidalis* Fabr.

2. Front and middle feet wholly black ...*portoricensis* Roeder
Front and middle feet yellow, first joint of the front ones black................................*[1]*grandiosus* Will.
All feet marked with white..................*rutilus* Coq.

---

[1] This species, of which I have never seen a specimen, probably belongs to the genus *Toxorhynchites*.—D. W. C.

IX.—GENUS ANOPHELES.

(*a*).—RECOGNIZED SPECIES.

1. With a yellowish-white spot near three-fourths the length of the front margin of the wing 3
   Without such a spot 2

2. Scales of last vein wholly black, palpi wholly black....... *maculipennis* Meig.
   Scales of last vein white, marked with three black spots, palpi marked with white at bases of last four joints..... *crucians* Wied.

3. Hind feet wholly brown, scales of last vein white, those at each end black................ ....*punctipennis* Say
   Hind feet largely snow-white on the apical half........... *argyritarsis* Desv.

(*b*)—UNRECOGNIZED SPECIES.

The following species which have been credited to our country have not been recognized with certainty; some of them probably do not belong to the present genus, while a few were evidently founded on badly rubbed specimens in which the distinctive characters were therefore wanting:

annulimanus v. d. Wulp. I strongly suspect that this does not belong to the present genus; the description applies fairly well to the male of *Culex consobrinus* Desv.

ferruginosus Wied. This name was proposed for the species previously described by Say under the name of *Culex quinquefasciatus*, but the description differs so decidedly from the one published by Say as to give the impression that it is founded on a different species. I strongly suspect that the type of *ferruginosus* is a rubbed example of *Anopheles crucians*, which was described from the same locality. Say's description of his *Culex quinquefasciatus* agrees very well with the species which I have identified as *Culex impiger* Walker.

maculipennis Meigen. According to Theobald this European form is identical with our *Anopheles quadrimaculatus* Say.

nigripes Staeger. This European species should be readily recognized by its unspotted wings.

albimanus Wied. Differs from our other species by the snow-white apices of the tarsi.

pictus Loew. It is evidently closely related to *crucians* Wied.

Our recognized species of Anopheles and their synonyms may therefore be listed as follows, the synonyms indented:

- *argyritarsis* Desv.
- *crucians* Wied.
  - ?*ferruginosus* Wied.
- *maculipennis* Meigen.
  - *quadrimaculatus* Say
- *punctipennis* Say
  - *hiemalis* Fitch

THE NEW CLASSIFICATION OF THEOBALD.

Mr. F. V. Theobald, the learned dipterologist of the British Museum, announces in the first two volumes of his vast and most interesting *Monograph of the Culicidæ of the World* (35) that a third volume will be required to complete the subject. He thinks that longer study will likely necessitate further subdivision of the present genera. He has finally selected the exterior scale-structure of the various parts of the body, particularly of the head, the mesonotum and the *scutellum* (the narrow tri-lobed plate lying between mesonotum and metanotum), as the most characteristic and persistent generic *differentia*. His conclusions, which are interesting and convincing, are best reproduced in his own summary (Vol. I, p. 95). I have made a few verbal changes in the paragraphs quoted in order to embody his latest observations:—

> The old genera I have retained are, then, *Culex*, *Anopheles*, *Aëdes*, *Megarhinus*, *Psorophora*, *Sabethes*, *Uranotænia* and *Hæmagogus*, and in a modified form *Tæniorhynchus* (for one of Arribalzaga's species in that genus and others I have observed) and *Janthinosoma*.
>
> To these I now add the following genera: *Wyeomyia* and *Deinocerites* (the former is separated on account of having *chætæ** on the metanotum, a character found in no other *Culicidæ* except the new genus *Trichoprosopon*, *Deinocerites* on account of the peculiar elongated form of the second antennal joint); *Aëdeomyia*,

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* Bristles.

separated from *Aëdes* on account of the broad wing scales; *Panoplites*, separated from *Culex* on account of the peculiar wing scales, somewhat like those of *Aëdeomyia; Eretmapodites*, with the hind tarsi densely scaled, forming a kind of paddle-like termination; *Janthinosoma* with peculiarly formed head scales, densely scaled hind legs and a pseudo-vein running through the first basal cell; *Stegomyia* and its sub-genus *Armigeres*, because of the curious flat-scaled or armor-like covering to the whole body, so strongly contrasted with the scale formation in *Culex* proper.

Closely related to *Psorophora*, yet clearly distinct, is the new genus *Mucidus*, in which the posterior cross-vein is nearer the apex of the wing than the mid-cross-vein and the wings covered with curious pyriform parti-colored scales, as well as having head scales of a form found in no other member of the family.

The genus *Toxorhynchites* contains but a single species, but is very marked, the wings being much as in *Megarhinus*, which it generally resembles, but the palpi in the female are three-jointed.

Amongst *Anopheles* there is no doubt that the species described as *A. Grabhamii* should constitute a new genus. Since I have received fresh material I have placed this pretty gnat in a distinct genus *Cyclolepppteron*. The genera *Stegomyia* and *Panoplites* are clearly defined from *Culex*, so is *Wyeomyia* from *Aëdes* and *Aëdeomyia*, *Mucidus* from *Psorophora*, and *Toxorhynchites* from *Megarhiuus*, but whether the others I have placed as genera,—*Eretmapodites* and *Armigeres*,—should rank as genera or sub-genera is a matter of opinion. *Eretmapodites* is clearly distinct.

Janthinosoma form a decidedly marked group, the hind legs having shaggy scales, and the hind tarsi being always white. *Armigeres* resemble *Stegomyia* in scale structure, but are much larger insects, and present quite different superficial appearance, and their larvæ differ. The genus *Trichoprosopon*, n.g., forms a most interesting group, in which not only *chætæ*, but *squamæ* are present on the thorax, and the clypeus,* etc., densely hairy.

Limatus, n.g., and *Brachiomyia*, n.g., are from St. Lucia and Brazil respectively. *Limatus* is closely related to *Sabethes*; *Brachiomyia* differs from all *Culicidæ* in the structure of the (female) antennæ.

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*A chitinous prolongation from the front of the head just above the proboscis.

CHAPTER V.

DISSECTION. SECTIONING. STAINING.

DISSECTION OF FRESH SPECIMENS.

This is far the quickest and in many regards the most satisfactory way of working with mosquitoes. For malarial experiments no microtome work should be tried till the student has completely mastered the difficulties of fresh dissection. At any time the general condition of a specially important insect (*i.e.*, whether infected or uninfected) is as a rule much more safely ascertained thus than with the microtome; though of course the study of the biological *minutiæ* requires sectioning.

The instruments urgently needed are few, though a larger equipment facilitates the work. A dissecting microscope is useful though not necessary; an ordinary hand lens, properly supported, may be used instead. Two fine dissecting needles are ordinarily employed, though personally I prefer and have used for a long time two sharp and small Graefe cataract knives. A fine forceps, a fine scissors, a small glass pipette or "dropper," and some clean slides and cover-slips should be provided; also a bottle of filtered sterile salt solution of strength six-tenths of 1 per cent. The bottle should be stoppered with cotton and the pipette sterilized before use; otherwise bacteria or spores will appear in the salt bottle in a few days, seriously confusing the field of dissection.

The mosquito is caught from the cage in a short test-tube or small bottle and killed with vapor of chloroform. Half a minute is usually enough. Some will revive if afterwards shaken about in the air,—a kind of "artificial respiration." If chloroform is not at hand, ether, benzine, tobacco smoke, or the cyanide bottle may be used. Legs and wings are cut off close with scissors.

If only the **stomach** is wanted, the abdomen is cut off close to the thorax and laid in a drop of salt solution on the slide. With the needles carefully detach the last two rings from those in front, taking care to touch only the extreme outside of each ring. Then pull the two detached rings backwards from the rest of the mass. By using great gentleness the ovaries and intestine will first appear and finally the Malpighian tubes and stomach. This method,—a very easy and pleasing one, originating, I believe, with Dr. Ronald Ross,—serves to show perfectly all the abdominal structures except the dorsal vessel, which does not bear on our studies. It must be worked out more slowly and laboriously with needles and forceps (Woldert, 13). Under the low power, before putting on the cover, the intestine, oviducts and neck of the stomach will be seen to be in active normal and reverse peristalsis, the contents of the stomach and gut flowing with each contraction backwards and forwards. The field will be at first obscured with turbid fatty fluid from the insect; this fluid must be carefully washed off with an excess of the salt solution till no cloud remains. The cover should not be put on (and then only with extreme care) till some of the broken chitine rings or one or two hairs from one's head are arranged to keep the cover from crushing the dissected viscera. In warm weather the salt solution under the cover must be constantly renewed during examination, or some pure (not carbolated nor rancid) vaseline run around the edge of the cover to stop evaporation. Such a dissection will stay good an hour or two, —longer if the gut was free of bacteria. Permanent preservation will be described later (p. 60).

In case one is studying large malarial coccidium-bodies, which are easily burst, it is better not to extract the stomach at once by pulling, as described, but first partially and very gently to break the under side of the chitinous sheath from the upper all the way along, and then draw out the stomach by pulling on the last two rings. Some experience will be needed before this can be successfully done in every case. The practice should be gotten with normal mosquitoes, so that one may be ready to work upon inoculated insects without disappointing failures.

To extract the **suctorial vesicles** and **œsophagus**, cut off the mosquito's head only; then pull on the last two abdominal rings as above, making counter traction with the forceps attached to the stumps of the front pair of legs. As a rule all the vesicles come away easily, —attached to the neck of the stomach. Giles (9), whose published description of this method is the only one I have seen, says that when the same method is followed without cutting off the head, the salivary glands, too, will sometimes come away. I can not confirm the observation,—even after repeated trial. Success evidently depends on whether the esophagus breaks from the proboscis behind or in front of the attachment of the salivary duct.

To extract the **salivary glands** the method recommended by the Italian observers (Grassi and others) reguires a very steady, well-trained hand and no little practice. The dismembered mosquito is laid on one side. The side of one needle is pressed gently upon the rear third of the thorax, while the other needle makes gradual traction on the head. In favorable cases the head comes away bringing both glands with it. They are apt to be bruised or broken in many cases; in still others the duct breaks before the glands appear. In this emergency, further pressure with the thoracic needle is recommended. In my own experience it pays better when one has a good dissecting microscope to try the following plan: Cut off legs, wings, head and abdomen; hold the thorax on one side in the dissecting fluid with forceps attached to the front pair of legs. With knife or needle carefully pick off the whole of the dorsal chitine in successive bits. Then pull away the wing muscles. The thoracic part of the alimentary canal and (after washing carefully) the salivary glands may be seen lying just above the first pair of legs. The glands may then be isolated. The cover should be put on with the same care already urged (p. 58).

PERMANENT PREPARATIONS.

Permanent preparations of fresh dissections cannot be made with any reagent I know of except with considerable sacrifice of the beautiful detail and comparative truthfulness of the preparations as they lie in normal salt solution. Fig. 49, drawn from a permanent preparation, may be compared with Fig. 36, made from the fresh dissection. Vacuolation and shrinkage of the cells and fibres seem unavoidable. The small malarial bodies are rendered sometimes almost unrecognizable and sporozoites in the glands can hardly be told from artefacts.—Two per cent. commercial formalin in $^{75}/_{100}$ per cent. salt water is recommended conditionally by Grassi. The fluid may be run under the cover gradually, then the excess wiped off and the cover ringed with Bell's or any of the good cements. Full details of cementing may be found in Lee's *Vade Mecum* (15). If a stain is wanted, the original dissection may be done in the fluid just named and a drop of picro-carmine added at one margin of the cover. Place the slide in a moist chamber 24 hours and add glycerine, finally cementing as before (Grassi). The stain is said to be often very successful.

One per cent. carbolic acid in glycerine (both reagents C.P.) may be applied to the margin of the cover in the same way. The mixture will gradually replace the water as the latter evaporates. The cover may be ringed for security as before, but the preparation will stay fairly good for some weeks without it. The action of this is rather variable. I have seen stomachs and salivary glands well preserved in it for a year or more; and on the contrary they sometimes "go bad" in a few weeks. The reason is probably to be found in the number of bacteria originally present in the mosquito's intestine.

Grassi suggests also a fluid consisting of the white of one egg, salt, grm. 1.50, and water, grm. 250. Beat and filter. The completed dissections are introduced into a drop of this fluid spread on the slide. They are then fixed in vapor of osmic acid and mounted in glycerine.

The dissected viscera may be transferred from salt water to concentrated solution of mercury bichloride, and after 2 hours into alcohol. Paraffin sections may then be made and stained or the preparation mounted at once in glycerine. Grassi (*l.c.*) thinks the cells well preserved by the latter method.

Serial sections of the entire mosquito, longitudinal or transverse, may be made and much may be learned in this way of the anatomy and pathology; particularly of the location of malarial sporozoites in the salivary glands.

The mosquito should be killed,—dead specimens spoil with great speed,—and dismembered. It should be dropped at once into 35 or 40 per cent. alcohol in a test-tube and heated to boiling. These are the usual directions. I have gotten good sections without boiling. Boiling expels the air completely from the tracheæ, and on cooling the fixing agent penetrates thoroughly. Change to 80 per cent. alcohol after a few hours, then 95 per cent., then absolute. The insect is easily,—too easily,—hardened, and the absolute alcohol should be used for a minimum of time,—say half an hour or so. The regular paraffin process is usually to be followed, though competent workers get good results with celloidin. I prefer paraffin of melting point 52° C. (125° F.), and use it all the year round. Though good observers say that this temperature injures the tissues, I have not myself observed it. In tropical climates the block, block-holder and knife should be cooled in ice-water before cutting. No special trouble need be encountered by skilled workers except the "desolating" fact that the chitinous sheath is apt to break and shatter the sections if it has been made the least bit too hard, or if it is cut with any but the sharpest knife. I formerly tried softening the chitine with *eau de Javelle* (Lee, 15, p. 472)*, but have latterly had good success without it by hurrying the preparatory steps, and using an extra-sharp knife. The Minot microtome cuts sections to a thinness of three microns,—which is

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*The processs is not difficult; after hardening, the mosquito is put into *eau de Javelle* for a few hours,—long enough to dissolve away the scales: then wash in water and again dehydrate.

thin enough for practical purposes. The paraffin ribbands may be laid on a sheet of clean paper, broken to convenient lengths and floated on a slide in a few drops of very dilute glycerine-albumin mixture. Giles (*l.c.*) recommends a drop to the watch-glass of water. The slide is warmed high over a flame till the sections completely unfold. The excess of fluid is then poured off and the sections dried at blood heat in the thermostat. The paraffin is then very gently washed off (with xylol, or naphtha, or turpentine), the albumin fixed with absolute alcohol, and the sections stained with weak methylene blue (2 per cent.) to which borax (5 per cent.) has been added (Manson's formula), or with hematoxylin and eosin.—Delafield's hematoxylin or "hæmalum" of foreign writers does equally well.

Giles recommends as a fixative 90 per cent. alcohol (2 parts) with solution of 1-1000 bichloride of mercury (one part), to be boiled as above.

Malarial sporozoites are most easily gotten by bursting the "ripe" malarial capsules in a drop of salt water as they lie on the surface of the stomach. The stomach must be freshly dissected on a slide. Dry the slide and cover slip (after removing the detritus), fix in absolute alcohol 15–25 minutes and stain with Goldhorn's blue* or with any one of the numerous Romanovsky formulæ† that has been tested beforehand. The chromatin is stained thus and shows well (Grassi). The sporozoites will stain with any fluid that stains the sporocytes or gametes well, *e.g.*, Jenner's stain. This also may be had (with directions) of any ot the large dealers in microscopic supplies. Manson's blue (p. 63) is also excellent. It requires only half a minute.

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*To be had of Richards & Co., 18th St., New York. Directions accompany the bottle.

†Perhaps the best of these is that described by Leishman (34). It may be had (with directions) of Dr. Grübler in Leipzig, or of his agents.

CHAPTER VI.

MALARIAL PARASITES.

The malarial parasites are now grouped in a single order, Hæmamœbidæ. They probably all have two modes of reproduction and bear very close biological relations to the *Coccidia*, many of which present a double cycle of evolution so closely similar to that of

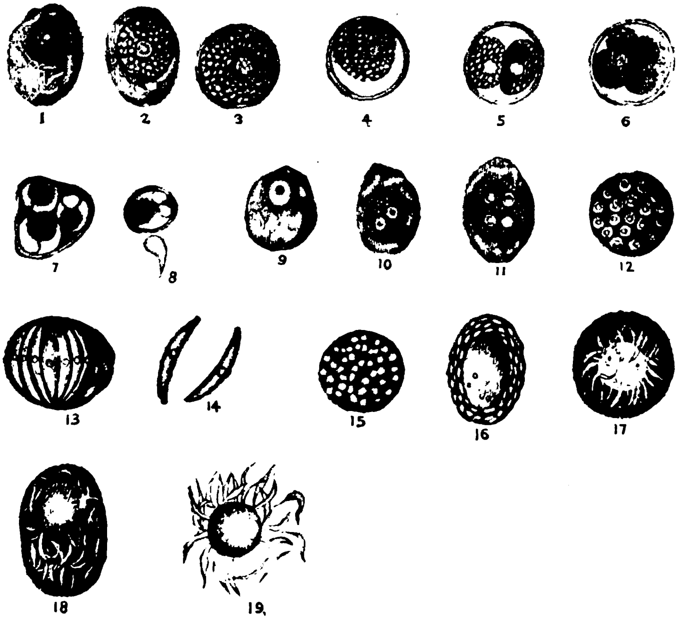

Fig. 41.—Reproductive cycles of *Coccidium salamandræ*. For general explanation see text. No. 8 is the exogenous spore just escaped from its capsule, and now capable of life exterior to the body of the salamander. No. 14 is the endogenous spore, dying at once after leaving the body of the host but capable in the intestine of infecting a healthy cell. Nos. 15-19 represent a rare variation of the endogenous cycle of development. Freshly mounted in a hanging drop the flagella are in active motion. (From Simond).

the malarial parasites as to be of absorbing interest. The double cycle of *Coccidium salamandræ* described by Simond (16) in 1897 is an excellent example. This parasite has a cycle completed in the stomach of the host (Fig. 41, Nos. 9-14), a cycle completed exterior to the alimentary canal of the host (Fig. 41, Nos. 1-8) and also a singular "pseudo-flagellate" type of development strongly suggesting the flagellation of the malarial parasite (Fig. 41, Nos. 15-19).

HUMAN MALARIAL PARASITES.

It is beyond the scope of this work to do more than mention the three species of malarial Plasmodia,—*tertian*, *quartan* and *estivo-autumnal*,—and touch on certain of their characteristics that are important in working with mosquitoes. In view of the half-dozen or more names proposed for these parasites, we use here only the most familiar.

Each parasite has in reality, as Manson (17) has well enunciated, 3 modes of existence:—that in human blood, that in the mosquito, and a "latent" intracorporeal (perhaps intravisceral) phase; the last being the cause of relapses of malarial fever. We are concerned here with the first two only.

Of these the parasite developing in human blood by periodic sporulation is called a *sporocyte;* that growing in the mosquito a *gametocyte* or *gamete*. The gametocytes of summer-autumn infections (fortunately for easy experiment) are marked by their characteristic crescentic (at times ovoid) outline (Figs. 42, 43). Those of tertian and quartan fever are to be told from large sporocytes only by minute differences in pigment, granulation and staining, and to know quickly whether tertian or quartan gametes are present in any special case the easiest way is to induce exflagellation.

Gametes are bisexual. The male is called by Ross (18) a *microgamete*, the female a *macrogamete*.* The former exflagellates, the latter incorporates one or more flagella into its substance and is thus fertilized (MacCallum, 26). Flagellation occurs only outside the human body, and is completed normally only in the stomach-wall of a specific mosquito (Anopheles) so far

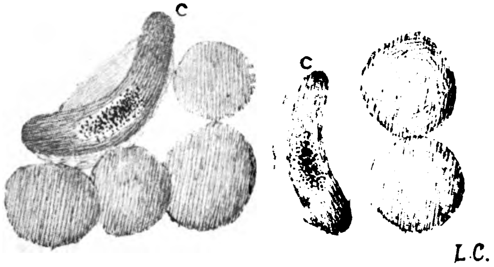

Fig. 42.—Blood cells of child infected with estivo-autumnal malaria; *c*, *c*, crescent bodies (gametes). (Original).

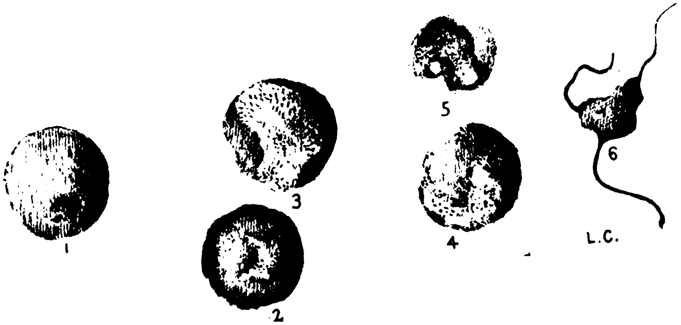

Fig. 43.—Tertian malarial parasites; 1, young sporocyte with chromatin dot; 2, 3, 4, 5, larger parasites; 6, drawn from a stained specimen of flagellated gametocyte. (Original).

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* Apart from the convenience of these names, it seems only a matter of historic justice that the epoch-making discovery of Dr. Ross should be thus recognized.

we now know. The female gamete is said to be distinguished from the male before flagellation by the slightly larger size of the former, the less active ameboid movement and coarser pigmentation. The details of the mosquito-stage are given on pp. 77-82.

MALARIAL PARASITES IN THE LOWER VERTEBRATES.

Excepting the parasites of birds (which are taken up briefly as a type) this fascinating subject cannot be enlarged upon here. The parasite (*Pyrosoma bigeminum*) of Texas fever in cattle is closely allied to the malarial parasites. Parasites have been found in the red blood cells of frogs (*Drepanidium ranarum*), turtles, birds, bats (19), monkeys (20), and many of the domestic *mammalia*. These parasites are, so far as the present knowledge extends, all different, and they are thought to find lodgment only in their respective hosts; but further experiment and research may yet prove that one or more of them bears important relations to human malaria.

Excepting Proteosoma of birds and Pyrosoma of cattle, their relations to mosquitoes or other blood-sucking insects are unknown. Dionisi (19), who has described three blood parasites in bats, was unable after many months of laborious experiment to transfer any one of the three to mosquitoes.

This field being now one of the most important within the range of scientific malarial studies, a few words on the blood parasites of birds are added, to facilitate the practical work on the same subject, which is described later.

Two avian parasites have been satisfactorily distinguished, — *Proteosoma* (Labbé), and *Halteridium*

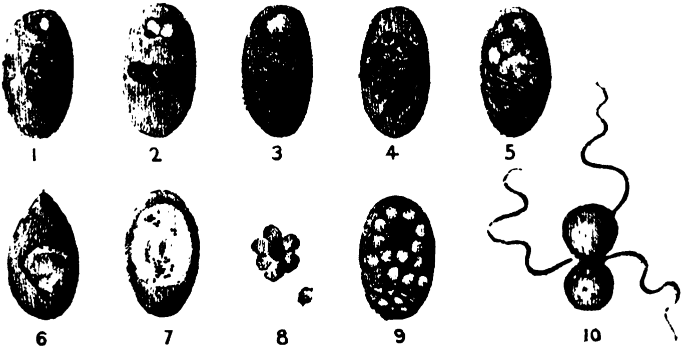

Fig. 44.—Proteosoma in blood of bird; 1-9, successive stages of the sporocytes; 10, flagellated gamete. (From Opie and MacCallum).

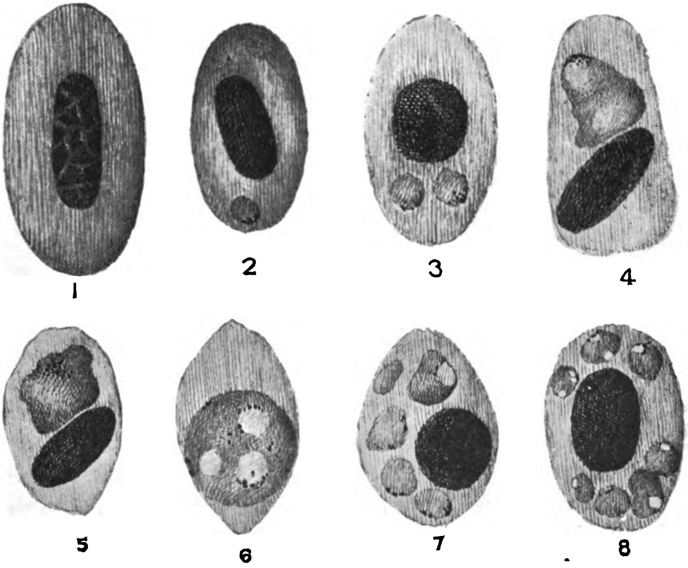

Fig. 45.—Stained specimens of Proteosoma in blood of English sparrow; 1, normal red cell (drawn out of scale; should be same size as 2); 2, young parasite; 3, double infection, nucleus out of normal shape; 4, 5, larger parasites; 6, full-grown parasite, nucleus absent; 7, 8, multiple infections found in blood cells from heart *post mortem*. (Original).

(Danilewsky).* The two resemble the malarial parasites of man in many essential regards. They have pigmented sporocytes and gametocytes, and the former develop and sporulate periodically in the red blood cells of the host. They may be easily distinguished from one another by the fact that Proteosoma displaces the nucleus of the red cell that harbors it, while Halteridium develops *around* the nucleus. The illustrations (Figs. 44, 45, 46) make these points clear enough. The gametes of Proteosoma have been found to develop in *Culex pipiens* (Ross, 22) and *Culex nemorosus* (Koch, 21). Neither of these mosquitoes is certainly an American

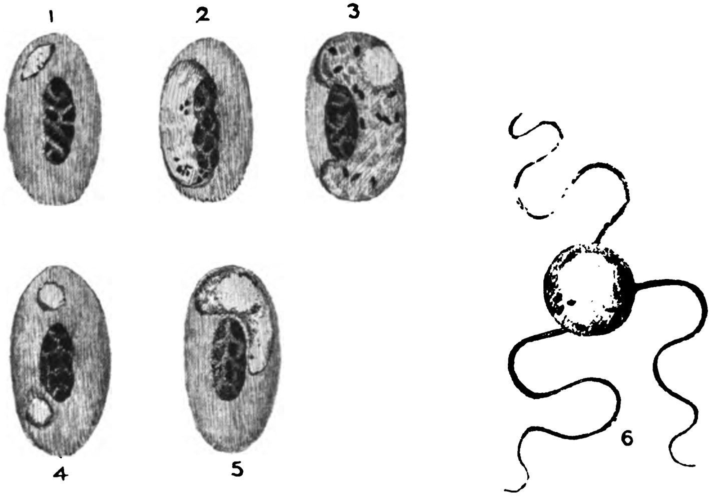

Fig. 46.—Halteridium in red blood cells of crow; 1, 4, young parasites; 2, 5, half-grown parasites; 3, full-grown parasite; 6, extracorpuscular flagellate body. (From Opie and MacCallum).

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*For simplicity's sake only the most familiar of the numerous synonyms are given.

species;* but from work of my own I am of the opinion that *Culex pungens* Wied. (the common American species) will also be found to carry Proteosoma. The insect host of Halteridium has not yet been found (Ross, 23).

PRACTICAL WORK.

Human Blood. — Fresh spreads from infected patients are, in my opinion, much the easiest means of identifying the malarial parasite. Directions for making them are to be found in any of the books on clinical microscopy. The secret of success is in having *perfectly clean slides and covers and perfectly fresh blood*,—the blood being drawn and mounted before it has time to clot. Only the oil immersion lens should be used. *Sporocytes* can be found—often in abundance—just before, during and just after a febrile paroxysm. Quinine soon expels them from the peripheral blood. If quinine has not been given, parasites may be found in minute numbers at any time between the paroxysms, but more pains are needed in the search.

In the malaria of the temperate zone *gametocytes* offer more difficulties. In summer-autumn cases crescents, if present, are usually easily found; but with us the crescents are often rare. As is well known they resist quinine for a long time, however, and may flagellate readily for days after quinine has been given. In tertian infections around New York flagellate forms are absent in a vast majority of the acute fresh cases that come to hospital. I have hazarded the conjecture (33) that either the gametes are more numerous in the peripheral blood at night or that these cases do not in-

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**C. pipiens* has been reported recently from America by Theobald.

fect mosquitoes at all. I have never been successful in repeated artificial inoculations (day or evening) of Anopheles with such patients,—even under the most favorable conditions. In the older, irregular, or relapsing cases gametes are usually present but often scanty. Workers nearer the tropics would probably not find this difficulty so great.

In favorable tertian cases (usually over two weeks old) flagellate organisms may often be seen in ordinary fresh spreads after 20 or 30 minutes in a warm room. Exflagellation may be hastened by Manson's suggestion (24) of spreading a minute drop of the blood quickly with a needle on a cover which has been just breathed upon; the cover (still wet) is put into a moist chamber of any convenient kind for 30 minutes. The wet cover may then be examined as a "hanging drop" (diluted with blood serum) or dried and stained by the method of the same author mentioned below.

If in searching for sporocytes fresh spreads cannot be used, dried and stained films must serve. The films should be fixed and stained soon after spreading if good pictures of the malarial chromatin are wanted. Before fixation the films keep fairly well for a long while in dry air. They should be protected from flies, and in very warm moist climates should be kept in glass-stoppered bottles with calcium chloride to prevent moulding (Koch, 25). The blood may be spread between clean cover-slips, or on slides. The method is familiar.

Stains are needlessly numerous. For those who need the information it may be stated that Jenner's, Goldhorn's, or Leishman's (modified Romanovsky) stains are as quick and practical as anyone usually needs (see p. 63).

To *stain the flagella* Manson's method is very pleasing and successful when flagellating bodies are numerous. A dozen or more covers are prepared *wet*, as on p. 72. After 20 to 40 minutes in the moist chamber they are dried high over a flame, fixed for 5 minutes in absolute alcohol and washed lightly in 5 per cent. acetic acid to remove the hemoglobin. After washing off the acid in distilled water, stain over night (6–8 hours) in 25 per cent. carbolic fuschin; wash, dry and mount. Dr. Manson has very courteously written me that this method succeeds perfectly at ordinary room temperatures* with crescent parasites *that flagellate easily*. Readiness of flagellation must be ascertained (it is a highly variable quantity) at the first, or much time may be wasted. The drawing on p. 67 (Fig. 43, No. 6) is from a flagellate tertian organism stained in the same way by myself.

Laboratory Work with Birds' Blood.—The importance of this work has only recently been again emphasized by Dr. Manson (17). The writings of Ross (22), Opie and MacCallum (26), and R. Koch (21) contain many valuable suggestions, also a bibliography.

Catching the birds.—Pigeons, doves and canaries may be had of bird-dealers in all cities of any size. Small wild birds can sometimes be gotten alive with nets or bird-lime. Crows (which are common carriers of Halteridium) may be caught in the nest when young or shot on the wing. Winged crows often live some days in captivity†. The barnyard fowls should not be forgotten; they have not received in the matter of malaria the attention they merit.

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*Martirano, quoted by Grassi (*l.c.*), states that the minimum temperature at which crescents flagellate is 74½° F. (17° C.).

† Dr. MacCallum informs me that most of his crows were gotten thus.

Much the most convenient bird, however, for ordinary experimental purposes is the English sparrow (Passer domesticus). There are no game laws against its destruction, and around New York in September* I have found Proteosoma infection in 75 per cent. or more of all sparrows examined. A third of all the cases were severe infections,—some with temperatures approximating 108° F. (Thermotaxis in birds, however, has not been carefully studied). In any region in warm weather where Culex is abundant Proteosoma-malaria is probably common in the sparrows. As sparrows are very suspicious and wary birds it is perhaps not too trivial to add the accompanying cut (Fig. 47) of a suc-

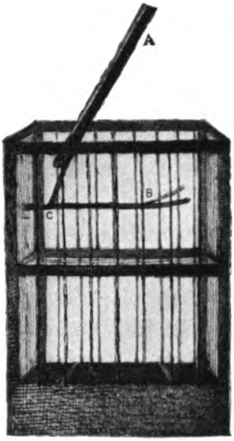

Fig. 47.—Bird-trap. Top of trap, A, revolves on a wire hinge passed through the upper edges of the sides of the cage, and shuts with a spring. A small strip extending from the lower edge of the top fits into a notch in the trigger at C. The fork, B, of the trigger supports a small tin-plate of oats. The lower compartment of the cage is separated from the upper by horizontal wires. In the lower compartment a live sparrow is kept as a decoy. The trap should be a little larger than an ordinary canary cage.

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* This is rather at variance with Koch's statement (21) that Proteosoma is confined to birds of "southern countries."

cessful sparrow trap, with description. The first live bird may be gotten with an air-gun. If the directions are carefully followed, this trap is infallible. The birds may be kept alive a long while in a well aired cage (*e.g.*, one for guinea pigs). Fresh water and oats and bread is all that they need.

Preparation and appearance of the blood.—Blood may be drawn from the wing (MacCallum and Opie), the pad of the claw (Manson), or from the thick pectoral muscle. The last is the only practicable place when relatively large amounts are needed for inoculation. For ordinary work the claw (the pulp of the last phalanx of the foot) is perfectly satisfactory. The area selected is cleaned with alcohol (and if feathered, clipped); a flat surgical needle should be used for the puncture and the blood spread with the usual precautions. When using the claw an assistant may be dispensed with if the bird is first firmly wrapped in a cloth in which holes for the feet are cut.

Uninfected birds may be inoculated in the pectoral muscle with infected blood from the same species. They sicken in 4 or 5 days when the technique has been correct. Canaries are easily infected thus with Proteosoma (Koch).

The appearance of a normal avian red cell is shown in the cut (Fig. 45, No. 1). If the spread was roughly made, many of the red cells will lose their nuclei, the latter floating about free in the serum with a little fine-granular matter attached. Crenation usually occurs just around the nucleus. Rouleaux are readily recognized. The various types of leucocytes correspond in many ways to those of the *mammalia* and need not be described. The polymorphonuclear forms have fusiform granules in the protoplasm.

The appearance of the malarial parasites will be well enough understood from the previous figures (pp. 69, 70). Ameboid movement is very sluggish in Proteosoma. Peculiarities in the pigment are well drawn in the cuts. Large extracellular clumps of it are often found.

Koch (21) commends the following method for observing flagellation in the case of Proteosoma or Halteridium:—Take 10 per cent. of avian blood serum, 90 per cent. of normal salt solution, and mix. Put a drop of this on a cover and add a minute fresh droplet of infected blood from a bird of the same species from which the serum was gotten. Flagellation is very rapid.

CHAPTER VII.

MORPHOLOGY OF THE MOSQUITO-PHASE OF THE MALARIAL PARASITE.—TECHNIQUE.

Nothing but a working outline is attempted here. The nomenclature of Ross is adopted. There are perhaps half a dozen other sets of terms, each with insistent advocates (Cf. Grassi, *l.c.*). The phenomena are so similar for all the known types of human and avian parasites that the following description fits all.

The *macrogamete* after fertilization (as already described) in the mosquito's stomach becomes an ovoid or elongated motile body, the *vermicule*. Stained sections of mosquito's stomach made 12 hours after a successful bite show the vermicules in quite large numbers,—some still in the stomach, some in the act of penetrating the stomach wall. In the course of 24 to 30 hours after the bite, the vermicule has usually pierced the epithelium of the stomach and attached itself to the outer muscular layer, the fibres of which it gradually dissociates as it increases in size. It is now a smooth, transparent, nearly colorless spheroidal body 5 to 8 microns in diameter and containing minute and irregularly arranged particles of black or brownish pigment. To this body Ross gives the name *zygote* (Figs. 48, 48A, 48B). It is well studied in fresh dissections, though *minutiæ* require staining. The number of zygotes depends on that of the gametes originally in the blood. Blood rich in crescent forms will produce dozens or even hundreds of young zygotes, though doubtless a minority of these come to maturity. The cut (Fig. 49) shows probably the average results of a bite from a moderate crescent infection. It will be seen that the zygotes collect mostly in the posterior two-thirds of the dilated part of the stomach. In

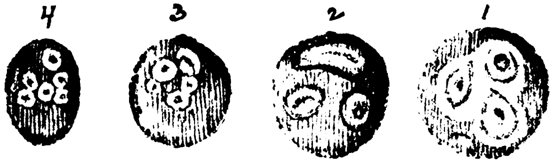

Fig. 48.—Young zygotes of estivo-autumnal fever in stomach-wall of Anopheles. Stained sections. (From Grassi).

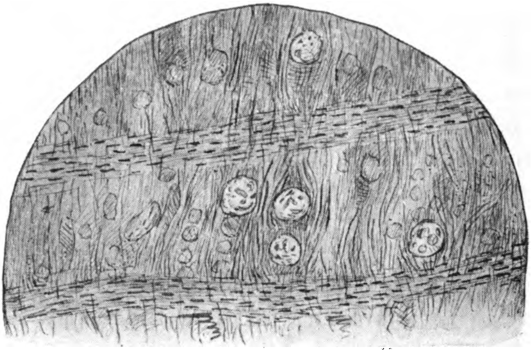

Fig. 48A.—From a preparation of mosquito's stomach, dissected 30 hours after the insect had fed on bird's blood containing Proteosoma. The pigmented cells or zygotes evidently lie between the longitudinal muscular fibers, which they have to some extent disassociated. (From Manson. Plate loaned by Messrs. Wm. Wood & Co.)

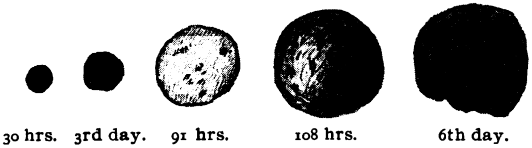

Fig. 48B.—Development of the pigmented cell or zygote in stomach of Proteosoma. The figure marked "6th day" is intended to represent what is evidently a capsule whose contents have escaped. (From Manson. Plate loaned by Messrs. Wm. Wood & Co.)

one or two instances I have thought I found one adhering to a Malpighian tubule where the tubes overlie the stomach. A few may be found, but seldom, growing on the narrow anterior part of the stomach.

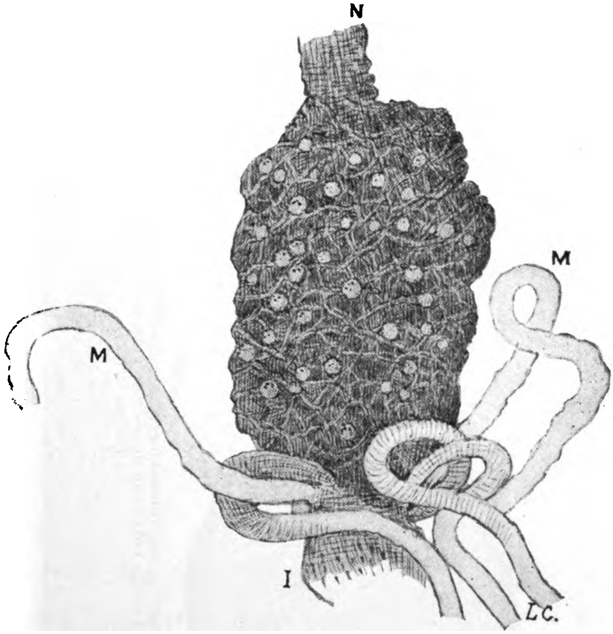

Fig. 49.—Stomach of *Anopheles maculipennis* showing malarial zygotes 4½ days old. The rounded pigmented zygotes lie in the meshes of the muscular fibres. N, neck of stomach; M, M, Malpighian tubules; I, ileum. (Leitz ⅔ds objective; specimen preserved in 1 per cent. carbolated glycerine. Original).

The zygote grows rapidly, and soon shows a visible capsule. In favorable conditions the capsule has attained by the sixth day a diameter of 40–60 microns (see Figs. 48-50, with legends) and is ready to rupture. Cold delays the process and temperatures below 24°–

25° C. (77° F.) are believed to arrest it completely (27). Between 85° and 95° F., the process goes rapidly forward. Starvation of the mosquito does not seem (Grassi) to prevent the ripening of at least some of the capsules.

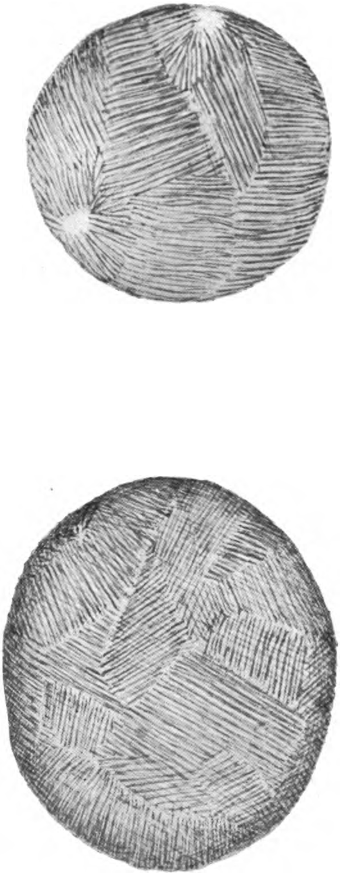

Fig. 50.—Mature zygotes. Right hand figure is optic section (oil immersion) of tertian zygote fixed with osmic acid. Left hand figure is zygote of Proteosoma, formalin and osmic acid. (From Grassi).

The young zygote is mononuclear. With the increase of the organism in size there is a continuous process of division of this nucleus till the number becomes enormously great (several thousand) and the size of the individual nuclei extremely small. These nuclei finally change into minute straight or slightly curved filaments (Fig. 50) grouped as in the figure, and easily visible through the capsule in the fresh dissection. When finally the capsule ruptures, the filaments—now 8-14 microns long—called by Ross *zygotoblasts*, by other observers *Sichelkeime* (Koch), *sporozoites* (Nuttall, Grassi), "filiform young" (Ray Lankaster),—are scattered in the *coelom* or general body cavity of the mosquito (Fig. 51). Left behind in the capsule are a few residual globules or irregular vacuolated masses, and a few zygotoblasts. The escaping filaments finally find their way (chemotaxis?) into the salivary glands. Any or all of the three lobules of each gland (p. 48) may be infected. The glandular ducts also usually contain filiform bodies in variable numbers.

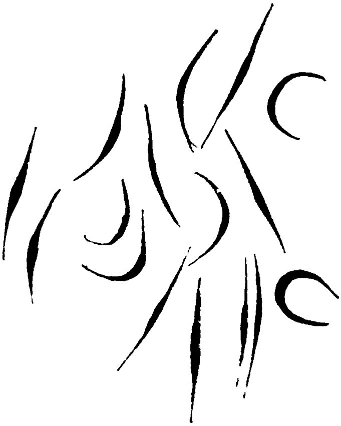

Fig. 51.—Zygotoblasts as seen in salivary glands of Anopheles; chromatin stained. (From Grassi).

A word should be added about the "black spores" of Dr. Ross's earlier writings. He now believes them to be degeneration forms. Grassi also figures them (Fig. 52) as such, and describes them as originating by the deposit of an *involucrum* of brownish or brownish-yellow matter around a sporozoite or around some of the "residual masses" already described.

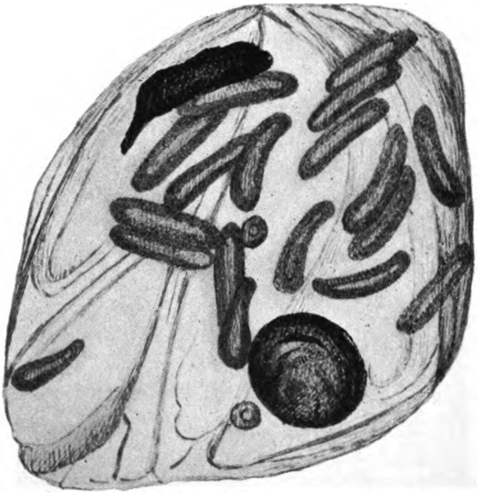

Fig. 52.—Brown bodies ("black spores") in ruptured sac of full-grown zygote, 11 days old. (From Grassi).

With the help of the descriptions and figures so far given workers should be able to find the rest of the way themselves. As already noted, the finer structure of the larger zygotes cannot be well studied except by staining (see p. 61). They may be readily seen with the ⅔ and ⅙ dry objectives. The small parasites should be studied with the oil immersion lens.

INOCULATION OF MOSQUITOES WITH MALARIAL PARASITES.

Many hints have been given already.

By preference **the mosquitoes selected for experiment** should be bred in the laboratory and should already have digested one meal of sterile blood. They should not be over a week old to insure their surviving the experimental bite as long as possible. If caught in the open they should be kept long enough for possible previous infections to have disappeared as "coccidia" from the stomach-wall. In such cases it should be remembered that of a "crop" of zygotes possibly already present in such a mosquito one or two may fail to develop as fast as others; care should be taken that such may not be confused with later experimental infections.

In warm summer weather mosquitoes will not bite oftener than every two or three days,—as soon, that is, as the last previous meal has been digested. To make them bite well starvation for a day longer helps. Experiments of any real value should be conducted at a temperature of 80° F. (27° C.) or over, and (as Giles has wisely remarked) preferably at that season of the year when the mosquito is normally most active. *Cæteris paribus* large mosquitoes, biting more blood, are more liable to infection than smaller ones. Of a number of mosquitoes biting a case of malaria at one time as many as 25 per cent. may fail for some reason to "take." Allowing for deaths and accidents besides, it is usually not worth while to conduct an experiment with less than half a dozen insects to start with; 20 are a good number.

Four or five separate cages of insects should be kept, each cageful to be fed on successive days so as always to have a certain number of hungry ones on hand. The time of day taken for the bite should, if possible, conform to the feeding habits of the mosquito in question. Anopheles, however. though normally crepuscular in its feeding habits, will bite in the day. If the light is very bright a cloth can be thrown over the cage when a bite is to be got.

The soft part of the patient's forearm may be laid along the side of the cage. The insects will usually bite quickly through the wire-gauze. Manson (18) says that Anopheles punctures a *moistened* skin more readily. In working with Psorophora and other mosquitoes not so far shown to be pathogenic in respect of malaria, a few Anopheles should be fed on the same patient at the same time, and the infection of some of the Anopheles should be certainly established before the conclusion be drawn that the other mosquitoes do not "take." This plan I devised for myself some time before I found it recommended by Grassi (*l.c.*).

In selecting a malarial case for bites it is useless, as already noted, to work with any which has not a reasonable number of gametes in the peripheral blood. The crescent cases are of course much the easiest in which to determine this fact.

In respect of birds not much more needs to be said. A small amount of ingenuity will meet all the difficulties. The mosquitoes should be turned loose into a wire-gauze bird-cage after the birds have gone to sleep; those insects that have bitten in the night may be removed early in the morning before the birds have time to eat them up. Sick birds of course do not require such precautions. Another plan (not so good) is that a thick cloth be placed over the cage to keep the birds quiet while the insects are within.

For the study of the stomach the student must be familiar with the anatomy of the part. One may begin 12 hours after the bite. In simply determining whether or not a given mosquito is infected the third or fourth day is the best time and fresh dissection far the safest and quickest method. For finer work stains and sections are needed. Chances of deception are not numerous. Epithelia if large may simulate small zygotes, but the larger epithelia are almost always "prickle-cells," and the "prickles" may be often made out with the oil immersion lens. The danger of prematurely bursting ripe capsules has been noted (p. 58). Mosquitoes suspected of infection and caught in the open should of course be examined also as to the condition of the lobes and ducts of the poison gland. No further instructions on this point are needed.

When a stomach to be examined is partly full of blood, the blood should be gently disengaged with the needle and salt water; nothing can be seen otherwise. When staining an entire stomach it has been proposed (Rees, *l.c.*; Marchiafava and Bignami, *l.c.*) to detach the epithelial membrane of the stomach in order better to see the muscular layer—the epithelia staining so deeply as to confuse the field. Much care and experience with the dissecting needles will be required to do this, though if it is successfully done the stained parasites show far better. It has been already suggested (p. 61) that picrocarmine may be used to stain the parasites without removal of the epithelial layer of the stomach.

CHAPTER VIII.

MOSQUITOES AND FILARIAL DISEASE.

A few words of introduction are needed. The **Filariæ** are nematode worms. Fifty or more species or varieties are described by Nuttall (29). In the recent beautiful and elaborate memoir of Annett, Dutton and Elliott (38) 212 species are listed from Stossich (40). Some are parasitic in the lower animals (birds, reptiles etc.), some in various regions of the human body. Only 4 or 5 (a sixth is doubtful) are proved thus far to bear definite relations to the blood or lymph of man (Manson, 17).

Of these it has been known since Manson's notable discovery in 1879 (32) that one, *Filaria nocturna* (*Bancrofti*) has a cycle of development in the mosquito's body. The difficulties at that day of keeping mosquitoes alive in captivity prevented Manson from further pursuit of his researches and the ultimate way of the parasite's return to the human system remained conjectural.

The impulse given to the study of mosquitoes by Ross's discoveries in 1898 turned the attention of Manson again to the subject, and from his laboratory in London G. C. Low (30) in 1900 was able to make the important announcement that he had found the fully developed *Filaria nocturna* in the proboscis of *Culex ciliaris*. Bancroft (37) had already foreshadowed this discovery in an earlier publication.

S. P. James (31) at nearly the same time made the same observations independently on *Culex microannulatus*, *C. albopictus*, *Anopheles Rossii*, and "other species of Anopheles."

Grassi and Noè (36) have more recently announced the conveyance of *Filaria immitis* from dogs to *Anopheles maculipennis* Meigen.

TECHNIQUE.

For full details of the biology and pathology of the human Filariæ the admirable account of Manson (17) should be consulted.

Of *Filaria nocturna* the adults are parasitic in the lymph passages of man. The eggs, after developing there to a certain degree and becoming enclosed in a transparent capsule, escape from the lymph-ducts into the blood as *embryos*. The embryo has been long known as "Filaria sanguinis hominis." Illustrations of it may be found in all the standard text-books. It appears in the patient's peripheral capillaries only after nightfall (8 to 9 p.m.). It may be found readily when present. A good-sized drop of blood is mounted under a cover-glass and examined with a low power dry lens. If the parasite is present in small numbers a dozen or more slides should be thus prepared. If the cover is ringed with vaseline the parasite will continue its violent motions for a week. Dried spreads may be beautifully stained with hematoxylin after fixing with alcohol or heat (Manson, *l.c.*). Contrast stains are also said to be successful. The hemoglobin may be removed with 5 per cent. acetic acid if the blood film is undesirably thick.

The study of the **mosquito-cycle**, as described by the writers mentioned, does not appear to be specially difficult except in the matter of keeping the mosquitoes alive in captivity long enough to complete the development of the Filaria.

Uninfected mosquitoes of the kinds named (probably many other species of Anopheles and Culex will "take") were inoculated from an infected patient in the evening or night. They were subsequently fed on "fruit juices" only, it being feared that the Filariæ might escape into any solid food-mass requiring to be punctured. This appears, however, to be a needless precaution (Manson).

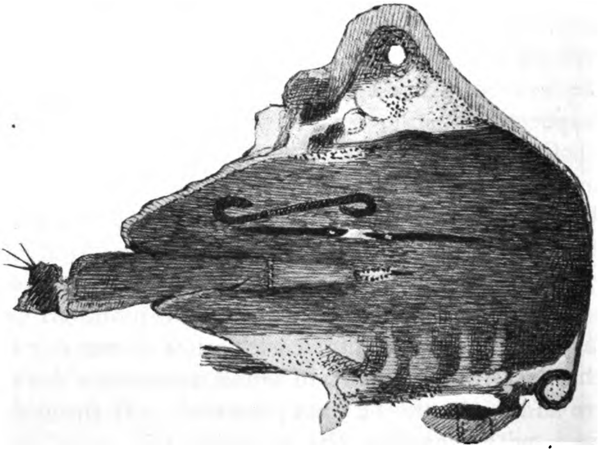

Fig. 53.—Stained section of thoracic muscle of *C. ciliaris*, showing *Filaria nocturna*; 11¾ days after feeding, development nearly complete. (From Low).

Examinations on successive days were made by celloidin sections (Low), or by teasing up the fresh thorax (James). The latter used plain water as a medium, but salt solution or blood serum would probably be better. He found the ⅔ and ⅙ dry objectives quite sufficient. Larger parasites when teased out were seen to wriggle vigorously for half an hour in water before dying. In blood on the slide they lived 7 hours.

For permanent preparations Manson (17) recommends the following:—The insects, preserved in glycerine, are soaked in 5 per cent. acetic acid one day, 50 per cent. formalin one day, absolute alcohol one day. They are then imbedded in celloidin (paraffin is undesirable) and cut in the usual way. The sections are stained in strong hematoxylin 2 hours, decolorized

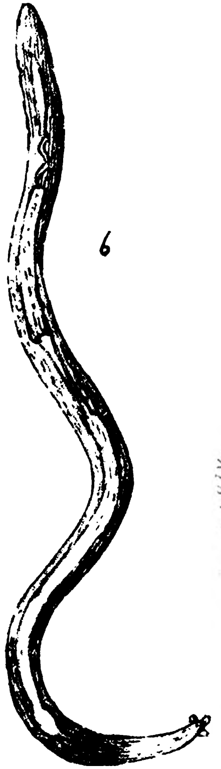

Fig. 54.—Filaria from *Anopheles Rossii*, eleventh day after feeding; showing separate parts of alimentary canal, tripartite tail, and differentiation of body substance to form reproductive organs. (From S. P. James).

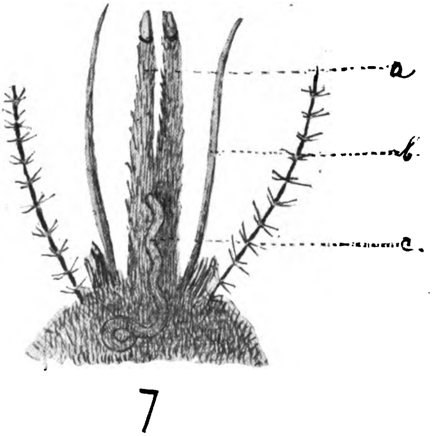

Fig. 55.—Proboscis and part of head of mosquito showing Filaria lying in tissues of labrum, *a*; *b*, mandible; *c*, Filaria. (From S. P. James).

with 1 per cent. HCl in alcohol, washed, cleared (xylol and anilin oil) and mounted in balsam.

To facilitate practical experiment a brief account of the mosquito-phase of the Filaria is added.

The **development** of the embryo of *Filaria nocturna* in *Culex ciliaris* has been described by Low (30) about as follows: In the insect's stomach the embryo first casts its sheath. Then quitting the stomach it penetrates the thick muscles of the thorax (Fig. 53). In these it passes through various changes, resulting in great increase of size and in the appearance of a mouth, gut and a "peculiar trilobed caudal appendage." The bulk of the young Filariæ finally accumulate in the prothorax near the salivary glands. A few atypically work back into the abdominal parietes. Those in the thorax pass along the connective tissue of the neck, enter the head, and (not piercing the esophagus as would be expected) bore a passage-way between the tough labium and the hypopharynx and work forward (head foremost, 2 nearly always together) among the stilets of the proboscis. The adjacent figures (Figs. 54, 55) from James further illustrate the process.

The worm is now of considerable size, measuring 1/15 in. (1.7 mm.) in length and about 1/800 in. (.033 mm.) in diameter (James).

No human being has been so far experimentally inoculated, but the analogy to malarial disease seems perfect, and there is the strongest probability that the bite of a mosquito thus infected will engraft the Filaria into the human system.

The **time** of the complete mosquito-cycle appears to vary between 10 and 20 days. Temperature and food doubtless influence the process, though in the same mosquito embryos simultaneously ingested are said to be found later in very varying stages of maturity.

CHAPTER IX.

MOSQUITOES AND YELLOW FEVER.

GENUS STEGOMYIA (THEOBALD).

By Aristides Agramonte, A.B., M.D.,
Professor of Bacteriology and Experimental Pathology, Medical School of the University of Havana, Cuba.

The comparatively recent demonstration of the rôle played by at least one of the species of this genus of mosquitoes in the propagation of yellow fever from the sick to the healthy non-immune, makes an apology unnecessary for presenting in this book a chapter dealing particularly with this newly created subdivision of the *Culicidæ*.

The name **Stegomyia** was suggested by the English entomologist, F.V. Theobald, who, while studying the insect known as *Culex tæniatus* or *C. fasciatus*, found that in many particulars it differed materially from the standard type of the genus in which it had been included.

Though according to this author but two species, *S. fasciata* and *S. signifera*, are to be found in the United States, I should be tempted to add one more to these, namely, the species now known as *C. tæniorhynchus* Wied., which in many features, it seems to me, is analogous to them; sufficient data have not, however, yet been collected to warrant this intrusion.—I would call attention to a previous effort on the part of Arribálzaga to establish a new genus with this species as a type, he probably recognizing the fact that it seems much out of place in the genus Culex.

My experience with this genus has been more or less restricted to one of the species, **S. fasciata**, the transmitter of the yellow fever parasite, I having been a member of the U.S. Army Medical Board, which clearly demonstrated this fact.

The most salient characteristics of this genus are (1) the palpi in the male, as long or nearly as long as the proboscis; in the female, the palpi are uniformly less than one-half as long; (2) the legs are destitute of erect scales; (3) the thorax is marked with lines of silvery scales.

AMERICAN SPECIES.

The species recognized as existing in the United States present the following features:

S. Fasciata Fabricius.

The front claws of the female (Fig. 56) are both toothed, one tooth on the under side; this is true only of one claw in the male (Fig. 57). In front of the wings, on the thorax, there is a more or less distinct outline of a lyre made up of silvery scales (the exterior outline much thickened) with the lower part of the instrument towards the head of the insect, so that there are four parallel lines (the two strings and sides of the figured lyre) upon the metanotum. The palpi of the male are sometimes even longer than the proboscis; those of the female are very short and are white at the tips. When held close together, these white spots give the appearance of a white band about the root of the proboscis, but the latter is not at all banded. The pleuræ are brownish with snow-white puncta. Abdominal segments with basal white bands, most distinct in front. Bases of tarsi are white.

S. Signifera Coquillett.

The front claws are destitute of teeth. The apices of the palpi are silvery white in the female. Thorax marked on the anterior half with 2 silvery white, subdorsal vittæ and with a pair of silvery white, arcuate

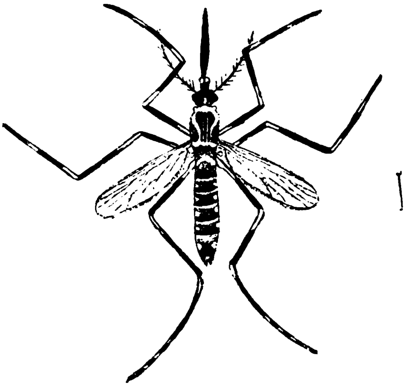

Fig. 56.—Adult female, *Stegomyia fasciata*. (Drawn by Dr. Agramonte).

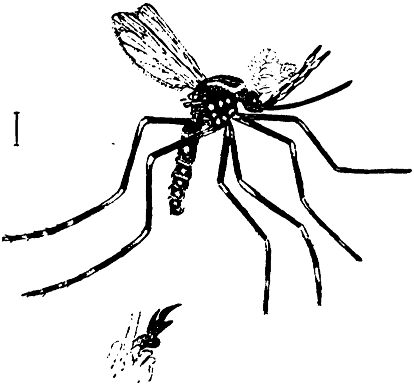

Fig. 57.—Adult male, *Stegomyia fasciata*. Claw of foreleg below. (Drawn by Dr. Agramonte).

lateral lines extending the whole length of the thorax. Pleuræ with several white puncta; abdomen black, whitish at the base of each segment; apices of tibiæ are white, also each end of the tarsal joints.

The exact reasons for separating these species from the genus Culex have not yet been published by Mr. Theobald*; it has only been hinted that it was on account of certain peculiarities of scale structure; we would add, also, the manner of oviposition, which is entirely different from that of Culex and not unlike that of Anopheles.

GEOGRAPHICAL DISTRIBUTION.

Stegomyia, or at least, *S. fasciata*, is spread over a wide range of territory, embracing many varieties of climate and natural conditions. It has been found as far north as Charleston, S.C., and south, as Rio de la

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*[It should be explained that Dr. Agramonte's manuscript was finished in November, 1901, and that the first copies of Mr. Theobald's vast *Monograph* (35) did not reach this country till January, 1902. Mr. Theobald's generic description of Stegomyia is as follows:

"Palpi short in the female, long in the male, four-jointed in the female, and five-jointed in the male.

"Head clothed completely with an armor of broad, flat scales; mesothorax covered with either narrow-curved or spindle-shaped scales; scutellum (the narrow trilobed plate between the mesothorax in front and metathorax behind) *always* with broad, flat scales to the middle lobe, and usually with them present on the lateral lobes; abdomen completely covered with flat scales, banded or unbanded, with white lateral spots. The female palpi are small, never more than one-third of the length of the proboscis; those of the male are long, or longer than the proboscis, and usually nude.

"Wings with similar venation to a typical Culex, but the fork cells short."

Mr. Theobald has described 17 species of Stegomyia from other parts of the world besides the 2 American species.—W. N. B.]

Plata. There is no reason to believe that it may not be present at some time or other in any of the intermediate countries. In the United States, specimens of *S. fasciata* have been captured in Georgia, Louisiana, South Carolina and eastern Texas. We have obtained specimens from Vera Cruz, Mexico, and from Jamaica, Curacoa and Porto Rico, W.I.; those from the last two islands were captured in winter. The finest sample of this species was sent to us, among many others, by Dr. D. B. Pelaez, from Rio de Janeiro.

The island of Cuba is well overrun with this insect; the city of Havana furnishes any number of individuals even in the winter months if they be sought for in the proper places. We have collected specimens from Santa Clara in June, Pinar del Rio and Guanajay in July, Quemados, Marianao and Columbia Barracks from January to December, until lately, that exterminative measures have been adopted and enforced. Many insects of this species have been sent to us from Baracoa, Santiago, Bayamo and Matanzas, and we do not doubt that a careful search would reveal its presence in all the larger cities of the Island, particularly, perhaps, along the coast.

The fact that *S. fasciata* has been known to exist at various times in Spain and other European countries may account for the spread of yellow fever which has occurred there once or twice in former times; the same may be said of the countries further north in the United States, where *S. fasciata* has not yet been reported but which have suffered from invasions of yellow fever.

LIFE HISTORY OF S. FASCIATA.

So soon as the perfect insect or imago comes from the water and dries, it takes flight, and in the majority of instances mates within a few hours; we have seen the female just rising from the empty pupa shell, still flying in the breeding jar, before alighting on its side, quickly joined by the male in the act of copulation, so we might say that immediately after birth they are already in a condition to propagate the species. Copulation occurs on the wing, male and female face to face. The male may repeat the function with 4 or 5 females in as many minutes. We have been unable to observe at any time any case of parthenogenesis, that is, in which unfecundated females have laid viable eggs.

The females of *S. fasciata* lay their eggs in the summer about 2 or 3 days after being fecundated, the fact of their sucking blood before oviposition seeming to bear no relation to this function. Dr. Findlay, a careful observer, and the originator of the mosquito theory in connection with yellow fever, for a time thought that the imbibition of warm blood was necessary to the insects for proper ovulation since they failed to lay eggs until after satisfying their bloody instinct. We have, however, obtained a goodly number of eggs from jars where we have never introduced anything more luscious than a slice of ripe banana, though eggs thus gotten were not as successful as those the parents of which had been supplied with their due ration of human capillary blood.

S. fasciata has been observed by me to lay her eggs as follows: The mosquito alighted upon the water, which was in a small beaker inside the jar, with legs spread wide apart. The abdominal segments being bent forwards and downwards, she dipped her whole body until the last segment touched the surface of the water; then she rose, walked a few steps, and dipped again. This she would do repeatedly (14 to 22 times), when she would remain for a slightly longer time with the last abdominal segment touching the water, and would allow a minute white egg to issue forth upon the surface. In this way she laid at the rate of 3 eggs per minute, resting quietly after every sixth or eighth egg for about 30 seconds when she would resume the process. This manner of oviposition has not been described I believe in any of the species usually discussed in connection with the infectious diseases. The eggs do not tend to approach one another. If bunched together in a test-tube they lie parallel.

Brackish water is unsuited for the development of Stegomyia larvæ. The species *S. fasciata* seems to select any deposit of water which is comparatively clean. The defective drains along the eaves of tile roofs are a favorite breeding place in Havana and its suburbs: indoors, they find an excellent medium in the water of cups of tin or china into which the legs of tables are usually thrust to protect their contents from the invasion of ants, a veritable pest in tropical countries. The same may be said of shallow traps where the water is not frequently disturbed.

Like other *Culicidæ* it prefers to lay at night. It is eminently a town insect, seldom breeding far outside of the city limits. I have never found *S. fasciata* resting under bushes, in open fields, or in the woods; this fact explains the well founded opinion that yellow fever is a domiciliary infection.

The **ova of S. fasciata** (Fig. 58) are uniformly black in color after laying, are conically elongated, and about 1 mm. long. The surface is finely checkered, like the stock of a gun, giving the egg an appearance of roughness, as though it were wrapped up in a finely reticulated membrane; this checkering of the surface allows the egg to retain minute bubbles of air attached to it, which very effectually contribute to its buoyancy. Any manipulation that will release these air bubbles will cause the eggs to sink to the bottom. At one time we thought that all eggs that sunk were irretrievably lost as far as subsequent hatching was concerned, but later experience has demonstrated that not only those that may have sunk but also that many that remain for one or two days dry upon the side of the water-vessel may become healthy larvæ. In fact, upon one occasion, a number of eggs obtained by our late deceased and much lamented colleague, Dr. J. W. Lazear, from Dr. Finlay had been deposited 30 days before upon the sides of the vessel, at the edge of the water; the level of the latter was raised by the addition of more water, the eggs becoming converted into larvæ soon after.

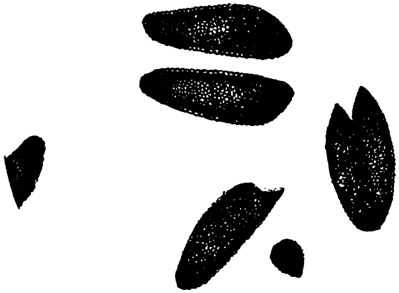

Fig. 58.—Eggs of *Stegomyia fasciata*. (Drawn by Dr. Agramonte).

A female *S. fasciata* may lay eggs to the number of 114 at one sitting, this being the maximum which we have observed, though the average may be said to reach only between 40 and 50.

The period of the egg-state varies according to (1) the season of the year; (2) the temperature of the water; and (3) the chemical condition of the latter.

The season of the year has a direct influence upon the rapidity with which eggs of *S. fasciata* hatch, regardless of the temperature of the water, the larvæ being born much sooner in the summer than in the winter months; this, no doubt, is principally dependent upon the condition of the parent insects at that season of the year. The hatching process is retarded upon cooling the water below 55° C. as is the case with other *Culicidæ.* Certain chemical conditions of the medium seem to favor or retard the development of the egg. In the lye of wood-ashes employed by laundresses in Cuba for the purpose of whitening the clothes, the eggs of *S. fasciata* produce larvæ sooner than in the dirty water of overflows or gutters, and yet the latter

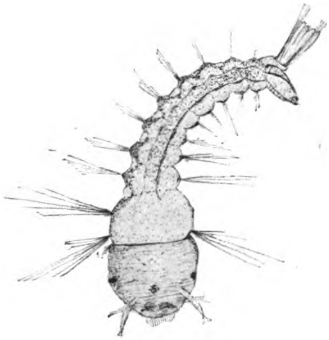

Fig. 59.—Young larva of *Stegomyia fasciata.*
(Drawn by Dr. Agramonte).

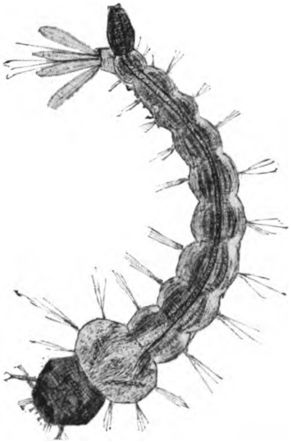

Fig. 60.—Full-grown larva of *Stegomyia fasciata.*
(Drawn by Dr. Agramonte).

contains a greater quantity of organic matter. The duration of the hatching process is therefore from 15 hours (the minimum observed) to 3 days.

The young **larvæ** (Figs. 59, 60) finally issue forth by the removal of a cap from the thicker extremity of the egg. The difference between the larvæ of this genus and those of Culex is slight; they both have equally well developed mouth-organs, with perhaps the advantage in favor of Stegomyia; the respiratory siphon, however, is shorter and thicker in the latter. As is the case in Culex, the larvæ have 6 Malpighian tubes while the imago has but 5. Three or four moultings are necessary before they reach full growth, which usually takes place at the end of 8 days in summer and 12 or 14 in winter. We have had 2 or 3 lots that failed to transform into pupæ in less than 20 days of larval life, but this is rather unusual with *S. fasciata*. The larvæ of this species seem rather sturdy in comparison with species of Culex; they skim rapidly along under the surface of the water and seem to feed voraciously. Dropping at short intervals to the bottom of the jar where there is abundance of Algæ and mould on the roots of the grass there placed, they remain without breathing through their siphons for an interval of even 4 minutes; this is the case particularly with the adult individuals; young larvæ remain much longer at the surface.

The question of hibernation in the larval stage is important; we have failed to get larvæ that could resist a freezing temperature and we can affirm that in the case of *S. fasciata* this degree of cold has proved uniformly fatal.

The possibility of their being capable of life outside their natural element must also be considered from an epidemiological point of view. The dry season in the countries where this species seems to abound is never so prolonged as completely to dry up the usual breeding places. In Cuba stagnant water in the neighborhood of houses is constantly being renewed by the overflow of defective plumbing and the usual carelessness of servants, so heretofore *S. fasciata* has grown and multiplied here with untrammeled freedom. Experimentally adult larvæ removed from the water and placed over night upon moist filter paper could not be revived the following morning.

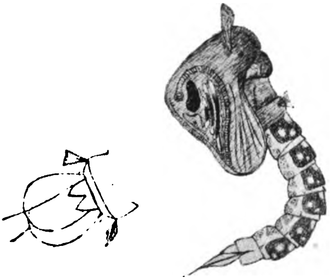

Fig. 61.—Pupa of *Stegomyia fasciata.* Anal flaps to left.
(Drawn by Dr. Agramonte).

The pupa of *S. fasciata* (Fig. 61) differs but slightly from the same stage in the life of Culex, showing only a lengthening in the thoracic portion and a greater thickness in the abdominal segments. The pupal stage occupies 2 or 3 days, after which the imago appears. We have therefore a developmental period at a minimum of 10 days. The usual ratio of males to females is as 1 to 6 or more; sometimes the reverse has occurred.

As to **internal anatomy** there is but one unusual condition that we have met with in several dissections; at the end of the oviduct in the Culex there is invariably present on each side a group of 3 very small, round, thick-walled glands with short ducts; these glands seem to be atrophied or barely outlined in the specimens of *S. fasciata* referred to.

The newborn fecundated female remains quiescent for 3 or 4 days; rarely can a *S. fasciata* be coaxed to bite before the fourth day; oviposition takes place at about this time.—Older mosquitoes have been seen to bite at intervals as often as 8 or 10 times.

The question of their **life-period** is of the greatest importance when we come to consider the apparently long interval which at times has occurred between the stamping out of an epidemic of yellow fever and its new outbreak without the introduction of new cases. The fact is that *S. fasciata* is a long-lived insect; one individual we kept in a jar through March and April into May for 76 days after hatching in the laboratory.

It was definitely shown by the experiments upon non-immune persons, that a period of at least 12 days at a temperature of about 83° F. (28⅓° C.) was necessary before the infected insect could transmit the germ of yellow fever from the sick; later on a mosquito which reached the age of 70 days in the hands of our colleague Dr. Carroll, was able to produce a case of yellow fever by stinging an American soldier 57 days after it became infected.

They succumb to the usual causes of death in mosquitoes. One cause proved most annoying to us on several important occasions, and that is, ants. In Cuba, at least, mosquitoes are often attacked by ants, probably while they sleep, or are sluggishly digesting a heavy meal, hanging on the walls. One or more of the little pests attach themselves to the mosquito's legs and there cling, until the insect, exhausted from continuous flying in an ineffectual effort to shake them off, falls to the ground and becomes an easy prey, or else it loses one or more legs, to die soon afterwards. During the experiments which the Army Medical Board referred to conducted last winter, we placed 15 infected mosquitoes in a room on December 21st. On the 28th of the same month only one mosquito remained alive; a couple of cadavers being carried away by ants told us the story of their disappearance. We have seen ants get into the jars kept in the laboratory so that it became necessary to isolate the counters and tables from the floor and walls. These facts, as well as others to be mentioned in connection with these experiments, can only be outlined here. For fuller details we must refer the reader to the Board's reports, published in the *Philadelphia Medical Journal*, October 27, 1900, and the *Journal of the American Medical Association*, February 16, 1901.

Adult Stegomyia feeds upon the same substances as Culex. The male undoubtedly supports its brief existence upon a Pythagorical diet. The female if opportunity offers sucks blood a few days after issuing from the pupa. She requires about 3 days for the thorough digestion of the blood and in winter a "good bite" often suffices for a week or more. Until digestion is complete it is absolutely impossible to coax the insects to sting again; at least this is the case with regard to those kept in the laboratory. When compelled to keep females for many days without blood-supply (having infected them from a yellow fever patient) we have found them to live very well upon over-ripe banana or other juicy fruit. They do not thrive upon sugar; it causes their legs to fall off or their feet to stick to the sides of the jars, though both males and females are inordinately fond of it.

They bite principally in the late afternoon, though they may be incited to take blood at any hour of the day.

They are abundant from March to September and even at the present date (November) we can capture them at will in our office or laboratory, both males and females.

I believe that Stegomyia is incapable of long flights unless very materially assisted by the wind; at any rate, the close study of the spread of infection of yellow fever shows that the tendency is for it to remain restricted within very limited areas, and that whenever it has traveled far beyond this the means afforded (railway-cars, vessels, etc.) have been other than the natural flight of the insect.

LABORATORY WORK.

S. fasciata is kept in the laboratory preferably in a large glass jar (dressing-jars, gold-fish globes, etc.) around the brim of which a bottomless bag of gauze is cemented with mucilage or attached by means of adhesive plaster. The mouth of the bag should close by a purse-string arrangement. A small receptacle with water (Petri dish, Stender dish, beaker, etc.) should always be kept in the jar for the eggs, also a few blades of moistened grass and a bit of fresh, quite ripe fruit. It is very simple to push the hand through the gauze into the jar and by means of a short test-tube remove any insect that may be desired for examination. For the purpose of inoculation the hand of the patient is thrust in (after removing the water and food) and the insects are allowed to gorge themselves, after which those that have not sucked blood may be easily transferred to another jar.

The life in the laboratory jar tends to produce decided alterations in the brilliant markings of these mosquitoes, particularly in the region of the thorax. They have a habit of flying with their backs against the sides of the jar, as though they would push their way out through the glass; thus many scales are rubbed off and the insects become almost unrecognizable.

To get them to bite it is necessary that the laboratory be kept at a minimum temperature of 80° to 85° F. The best hour for infecting mosquitoes is the afternoon or early evening, although we have been able to do so even in the early morning, a time of day in which they appear to be most sluggish.

Subsequent to the bite of the yellow fever patient the same temperature must be maintained for at least 12 days in order to enable the parasites to perform the evolutions in the body of the mosquito that will render them capable of reproducing the disease. In winter insects kept at this temperature have failed to infect even after 18 days.

Experiments have demonstrated that not all the mosquitoes which bite a yellow fever patient become infected, but that of several which bite at the same time some may fail either to get the parasite or to allow its later development in their body; it is important to bear this in mind when considering failure to reproduce the disease. The same is true of Anopheles with regard to malaria. On the other hand, when properly infected, the bite of one insect has been shown to produce a moderate attack of yellow fever.

How long do infected mosquitoes remain dangerous to the non-immune community? This question cannot be definitely answered at present; there is good presumptive evidence that the mosquito may harbor the parasite through the winter and be enabled to transmit in the spring an infection acquired in the fall. We have said that a mosquito produced a clear case of yellow fever 57 days after it became infected; there is reason to believe that the mosquito, once infected, can transmit the disease at any time during the balance of its life.

With regard to the parasite itself, we cannot as yet say much. Numberless dissections of infected insects have been made (the technique is exactly the same as for other *Culicidæ*) and serial stained sections have been prepared at various periods. The latter have led us to expect something tangible in the near future, as we believe that in the venomo-salivary glands of some of our specimens we have seen unusual conditions not present in the uninfected individuals. In this work the writer has obtained the efficient coöperation of Dr. W. E. de Salazar, Chief of the Histological Laboratory, who is exceptionally expert in the delicate manipulations necessary. He prefers to use paraffin for embedding; his sections are cut with a Minot microtome.

The question whether other genera of the *Culicidæ* or other species of Stegomyia are capable of transmitting yellow fever is still open for discussion. Work in this line of investigation will no doubt be undertaken after much that is still in obscurity is cleared up. It is best not to forget that such a thing is possible, though personally I am inclined to think that yellow fever is restricted for its propagation to the genus Stegomyia.

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